

VOL II

# Estudos em Ciências Agrárias e Ambientais

Eduardo Spers  
(Organizador)



EDITORA  
ARTEMIS

2024

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## APRESENTAÇÃO

O campo das Ciências Agrárias e Ambientais desempenha um papel fundamental na compreensão e solução dos desafios contemporâneos relacionados à produção de alimentos, à conservação ambiental e ao bem-estar animal. Em um mundo em constante transformação, questões como a sustentabilidade dos agroecossistemas, o manejo eficiente dos recursos naturais e a saúde pública se tornam cada vez mais relevantes. É com este espírito que apresentamos o volume II da coletânea "Estudos em Ciências Agrárias e Ambientais", que reúne pesquisas de autores de diversas partes do mundo, cada um contribuindo com sua perspectiva e expertise únicos.

Os quinze artigos que compõem este volume abordam uma variedade de tópicos, refletindo a riqueza e a diversidade das Ciências Agrárias. Desde práticas conservacionistas que buscam melhorar e manter agroecossistemas, até investigações sobre o uso de fitohormonas e fertilização na produção vegetal, o uso de tecnologias de processamento de madeira e a promoção do bagre armado - cada estudo traz à tona questões cruciais que impactam tanto a produção agrícola quanto a saúde ambiental.

Neste volume, também exploramos a crescente relevância dos produtos agrícolas locais, especialmente em tempos desafiadores como os que vivemos, marcados pela pandemia da COVID-19. A importância de circuitos curtos de proximidade se torna evidente, promovendo não apenas a segurança alimentar, mas também a resiliência das comunidades.

Além disso, as contribuições da veterinária destacam a importância do cuidado animal e da saúde pública, ilustrando a interconexão entre os seres humanos, os animais e o meio ambiente.

Esperamos que esta coletânea não apenas informe, mas também inspire debates e colaborações futuras entre pesquisadores, profissionais e estudantes da área. Juntos, podemos avançar em direção a um futuro mais sustentável e equilibrado, em que conhecimento e pesquisa sejam os pilares para soluções efetivas.

Agradecemos a todos os autores e colaboradores que tornaram este trabalho possível. É nossa esperança que os estudos aqui apresentados contribuam para um entendimento mais profundo das questões agrárias e ambientais, e que possam servir de base para novas investigações e práticas inovadoras.

Eduardo Eugênio Spers

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# CAPÍTULO 1

## PRÁTICAS CONSERVACIONISTAS PARA MELHORIA E MANUTENÇÃO DOS AGROECOSSISTEMAS

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**RESUMO:** Com a acentuada redução dos recursos naturais e as altas e crescentes demandas por alimentos, cada vez mais, torna-se necessário e vital a preservação dos agroecossistemas ou ecossistemas de cultivo. O presente trabalho buscou apresentar práticas agrícolas conservacionistas e sustentáveis, de forma a manter um equilíbrio entre produtividade agrícola e qualidade ambiental. Alguns modelos agrícolas como o sistema de plantio direto, preservam a biodiversidade dentro e sobre o solo, além de

proporcionarem uma série de benefícios para o ambiente, com resultados, inclusive, de altas produtividades de *commodities*. Rotação de culturas, adubação verde e a percepção de valor ao utilizar manejos sustentáveis, com o objetivo de preservar o solo e cuidar de sua qualidade devem fazer parte do dia a dia do produtor rural, uma vez que essas práticas influenciam, diretamente, toda a cadeia do agronegócio até atingir o consumidor final.

**PALAVRAS-CHAVE:** Preservação. Biodiversidade. Produtividade. Manejo. Sustentabilidade. Plantas de Cobertura.

### CONSERVATIONIST PRACTICES FOR THE IMPROVEMENT AND MAINTENANCE OF AGROECOSYSTEMS

**ABSTRACT:** With a marked reduction of the natural resources and the high and growing demands for food, it becomes increasingly necessary and vital to preserve agroecosystems or farming ecosystems. The present work aims to present conservationist and sustainable practices, maintaining a balance between agricultural productivity and environmental quality. Some models of agriculture, such as no-tillage system, preserves biodiversity in and on the soil, in addition to providing a series of benefits to the environment, with results including high productivity of commodities. Crop rotation, green manuring and the perception of value in using sustainable management, with the aim of

preserving the soil and taking care of its quality, must be part of the daily life of the rural producer, since these practices directly influence the entire agribusiness chain until to reach the final consumer.

**KEYWORDS:** Preservation. Biodiversity. Productivity. Management. Sustainability. Cover Crops.

## 1 INTRODUÇÃO

A população mundial alcançou 8 bilhões de indivíduos em 2022, e projeções indicam que em 2050 chegue a 9,7 bilhões (ONU, 2019). O aumento populacional, implica em uma demanda crescente por alimentos, tanto em volume quanto por qualidade. De forma a assegurar essa demanda, sistemas produtivos viáveis, eficientes e sustentáveis são essenciais.

O cenário do agronegócio brasileiro passa por notáveis transformações socioeconômicas, culturais, aperfeiçoamentos tecnológicos, estudos e pesquisas em diversas áreas do conhecimento, mudanças ambientais em evidência e alterações mercadológicas frequentes. Essa dinâmica envolve diversos setores, bem como direcionam a forma como toda a cadeia de produção de alimentos deve se comportar, a qual depende, inicialmente, do setor primário e da produção das matérias-primas.

Uma das principais funções exploradas do solo é a produção de alimentos e suprimentos de matérias-primas, como fibras e energia entre outras, para produções agroindustriais. Uma vez que o solo é utilizado, torna-se fundamental ações para promover sua preservação e sustentabilidade. Algumas técnicas agrícolas têm se destacado no quesito conservação e sustentabilidade dos capitais naturais, como o solo. Dentre elas, tem-se as práticas conservacionistas, que se apresentam como uma forma de melhoria e conservação dos agroecossistemas, os quais influenciam na cadeia do agronegócio.

No Brasil se observa que os agroecossistemas passam por produções contínuas, em que as plantas exercem um papel importante na ciclagem e fornecimento de nutrientes aos solos e na manutenção da sua biodiversidade. Por sua vez, a biodiversidade coexiste associada à esta população vegetal cultivada, recebendo os benefícios e impactos dos insumos e do manejo, que devem ser adequados ao ambiente em que se encontram.

Neste contexto, as práticas de manejo e as ações conservacionistas são fundamentais para preservar o ambiente como um todo. O sistema de plantio direto (SPD), a rotação de culturas, a adubação verde são algumas das práticas saudáveis de conservação e preservação, que geram inúmeros benefícios. Dentre eles, estão o aumento dos teores de carbono no solo, aumento da fertilidade, preservação física,

química e biológica de ambientes agrícolas, e aumento da produtividade e rentabilidade dos agricultores.

Dessa forma, o objetivo deste trabalho foi apresentar conteúdos sobre algumas práticas conservacionistas as quais podem possibilitar melhorias em diversos aspectos, bem como a conservação dos agroecossistemas. Os conteúdos serão apresentados de forma geral sem a intenção de esgotar o tema.

## 2 DESENVOLVIMENTO

### 2.1 DEFINIÇÕES E QUALIDADE DO SOLO

É possível encontrar alterações desfavoráveis em alguns elementos que compõem o solo, possíveis de serem percebidos à medida que os anos passam, quando a decisão de manejar os solos é decidida, exclusivamente, por fatores econômicos e não pensando em sua preservação. Sob estas premissas, são observadas mudanças na composição do solo, como por exemplo danos a sua estrutura e perdas de carbono do solo (Lobo Jr et al., 2022). Entende-se que a matéria orgânica é importante para colaborar com a fertilidade do solo e esta é medida pela presença de carbono no ambiente.

Um dos desafios para a agricultura sustentável é a mudança na forma de produzir alimentos. Para superar o desafio do aumento de produtividade, simultaneamente, à manutenção agrícola sustentável, existe um conjunto de práticas conservacionistas, como alternativas potenciais para reduzir os impactos ambientais das atividades agrícolas (Fortini, Braga e Freitas, 2020). Assim, identificar e introduzir formas para desenvolver uma agricultura mais sustentável e produtiva torna-se fundamental.

Para que haja resiliência nos solos, pode-se mencionar os solos supressivos, os quais são definidos como meios de monitoramento biológico conservativo de doenças, quando opositores e outros micro-organismos próprios do meio devem ser preservados e ativados, promovendo a supressão e a saúde nos agroecossistemas como um todo (Eilemberg et al., 2001). Entende-se, que suas principais características foram definidas por Baker e Cook (1982), onde pragas ou doenças e deficiências das plantas se estabelecem mas não persistem, causando baixo ou nenhum nível de dano. A severidade ou incidência de doença se estabelece em nível baixo e perde a importância (Lobo Jr et al., 2022).

Há formas de promover a supressividade e proteção de agroecossistemas, utilizando avaliações da qualidade do solo, que podem fornecer dados para as tomadas de decisões, com o objetivo de manter e devolver para o solo o que dele foi exportado (Lobo

Jr., et al., 2022). A tabela 1 mostra alguns conceitos de atividades agrícolas que podem ser úteis para promover essa supressividade e promover a proteção dos agroecossistemas.

A qualidade do solo em sistemas agrícolas refere-se à capacidade continuada de mantê-lo equilibrado sob o ponto de vista físico (aeração, retenção de água, compactação, estruturação), químico (reação do solo, disponibilidade de nutrientes) e biológico (teor de matéria orgânica, respiração, biomassa de carbono e de nitrogênio, taxa de colonização e de espécies de micro-organismos), de forma a sustentar processos e funções que proporcionem um ambiente favorável para o crescimento das plantas (Petrere e Cunha, 2010).

Os solos com boa qualidade são resilientes a estresses ambientais e proporcionam maior estabilidade de produção ao longo do tempo (Cherubin et al., 2017).

Tabela 1. Algumas definições.

<b>Termos</b>	<b>Definições</b>	<b>Autores</b>
Solo supressivo	Solos onde a ocorrência de patógeno não permanece ou, com sua presença, causa baixo ou nenhum tipo de dano. A gravidade ou ocorrência de doença classifica-se como baixa e com pouca importância, pois os patógenos não atingem o seu potencial que prejudicam o ambiente	(Baker et al., 1982)
Qualidade do solo	A classificação de um solo de se estabelecer em limites do ecossistema natural ou sob manejo, em manter a produtividade vegetal e animal, assegurar ou promover melhorias da água, do ar e apoiar a subsistência humana	(Karlen et al., 2003)
Saúde do solo	Traz referência à qualidade do solo que, segundo Doran et al., (2013), é a habilidade do solo de se manter como um sistema gerador de vida, operando dentro das demarcações do ecossistema, para promover a capacidade produtiva biológica, manter a qualidade do ar e da água e promover a saúde das plantas, a saúde animal e, também, humana	(Doran et al., 2013)
Agricultura regenerativa	Há uma reprogramação do sistema para recuperar a base de recursos por meio de aumento e melhorias da biodiversidade. Esta reprogramação são serviços ecológicos naturais, com alvo principal de promover a saúde do solo ou recuperar o solo degradado, com impacto no ambiente com menor intensidade e positivamente.	(Rhodes, 2017)
Práticas de agricultura conservacionista	Uma série de práticas agrícolas, onde a busca por produtividade integra solo, água e biodiversidade, com foco na preservação e menores impactos negativos ao ambiente	(Denardin et al., 2012)

No dia a dia da agricultura, pode-se observar agentes de (I) promoção da qualidade do solo e de (II) degradação do solo (Cherubin et.al, 2022).

Como agentes de promoção da qualidade pode-se mencionar as seguintes práticas:

(i) ajuste do pH do solo, pela calagem, a cada ano ou a cada dois anos, conforme a necessidade apresentada. O calcário (contém carbonato de cálcio e magnésio), é utilizado como corretivo do solo, tem objetivo de neutralizar o alumínio, corrigir a acidez com elevação do pH e melhorar a disponibilidade de nutrientes para as plantas. Sua ação se dá na camada superficial do solo, devido sua baixa mobilização. É necessário fazer coleta do solo em questão, nas camadas de 0-20 cm, enviar para análise em laboratório especializado, para posterior levantamento de dados, a fim de fazer a recomendação de aplicação de calcário adequada.

(ii) aplicação de gesso agrícola, pela gessagem, possui maior mobilidade no ambiente das plantas, não altera o pH do solo, diminui a atividade do alumínio em solução, fornece cálcio e enxofre nos perfis mais profundos. É necessário coleta do solo nas camadas de 20-40 cm, análise em laboratório especializado, para posterior análise de dados, a fim de indicar a quantidade de gesso a ser aplicado (Vitti et.al, 2015).

(iii) adubação equilibrada ou a adubação recomendada conforme a necessidade, trata-se de um processo que precisa ser aplicado no ambiente ou um processo de reposição de nutrientes ao longo do tempo. Dentre as variáveis para o bom desenvolvimento das plantas, pode-se mencionar os nutrientes. Se os mesmos estão disponíveis, pode-se observar um desenvolvimento saudável das plantas e boas produtividades.

(iv) controle de tráfego, com objetivo de prevenir a compactação do solo em subsuperfície.

(v) produtos biológicos, como bactérias e fungos, microorganismos destinados ao controle de pragas e doenças na agricultura (Embrapa, 2019); dentre outros agentes que promovem a qualidade do solo.

Como agentes de degradação do solo tem-se as práticas: (1) baixa produção de palhada; (2) compactação do solo por tráfego; (3) falta de nutrientes; (4) uso indiscriminado de defensivos agrícolas; (5) erosão, dentre outros (Cherubin et.al, 2022).

Diversos estudos estão voltados para a capacidade regenerativa e resiliente do solo e das plantas, mas ainda assim, há muito a se pesquisar. As variáveis ambientais como a precipitação, temperatura e umidade do solo, interferem na saúde e qualidade do solo (Raza et al., 2019). Mas, quando se observa que microrganismos têm pelo menos uma parte de seu período de vida colonizando a matéria orgânica, a oferta de raízes em determinados períodos do ano e restos culturais nos solos é um fator nutricional importante que afeta a sua capacidade regenerativa nos diferentes ambientes. (Lobo Jr. et al., 2022).

Na agricultura brasileira se observa a aplicação de técnicas de manejos sustentáveis, como as práticas conservacionistas. Entende-se, então, que a agricultura conservacionista é uma série de práticas agrícolas, onde uma vez que há o manejo integrado do solo, da água e da biodiversidade procura-se ter produtividade com menores impactos ao meio ambiente (Denardin, 2012).

É relevante apontar estudos sobre diversas formas de produção e seus impactos ambientais, sociais e econômicos, para que se saiba lidar com os pontos críticos e os planejamentos agrícolas. Práticas de manejo conservacionista para melhorias e manutenção dos agroecossistemas são vitais para a subsistência do ambiente e para a produção de alimentos, com demanda cada vez maior.

## 2.2 AGROECOSSISTEMAS, PRÁTICAS CONSERVACIONISTAS E SUSTENTABILIDADE

Os princípios de um agroecossistema sustentável são vistos na preservação dos recursos naturais, na adequação dos cultivos ao ambiente e na atenção ao manejo do solo em um nível moderado e não intenso. A produtividade sustentável ocorre quando há um equilíbrio entre solos, plantas, nutrientes, umidade, luz solar e outros organismos do ambiente (Altieri et al., 2002). Um agroecossistema com um meio produtivo saudável, é aquele onde se mantém condições de desenvolvimento de plantas de forma equilibrada, para que estas suportem estresses e adversidades (Altieri, 1998).

Práticas que preservam o solo estão associadas ao manejo sustentável e ao aumento da produtividade agrícola. Ao utilizar dessas práticas, pode-se observar resultados como a atenuação da erosão, redução da compactação do solo, a regulação térmica, aumento dos teores de matéria orgânica, promoção e preservação da biodiversidade (Altieri, 1998).

Como exemplo dessas boas práticas tem-se que ao se introduzir as plantas vivas de cobertura ou para formação de palhada, pode-se esperar resultados multifuncionais, dentre eles, a proteção física da superfície do solo, o aumento do carbono, a ciclagem de nutrientes além de colaborarem para a preservação do ambiente, esses impactam nos fluxos de ar, água, nutrientes e no crescimento radicular das plantas (Altieri, 1998).

O manejo conservacionista é uma forma sustentável de manejo, refere-se a um conjunto de técnicas agrícolas de menor impacto ao solo (Embrapa, 2022). As práticas conservacionistas inseridas nas ações de manejo do solo podem promover melhorias dos nutrientes disponíveis para as plantas, fertilidade do solo e resultados mais produtivos das lavouras implantadas.



Pode-se observar que os sistemas conservacionistas de produção agrícola são alternativas que buscam sustentabilidade na agricultura, proporcionam cuidados relevantes para a preservação da qualidade do solo, intensificam as atividades produtivas, gerando rentabilidade e alimentos para a população.

### 2.3 SISTEMA DE PLANTIO DIRETO (SPD)

Os primeiros registros do SPD datam de 1950. Nesse período, agricultores da Inglaterra e dos Estados Unidos da América implantaram experiências utilizando essa técnica que, na época, foram vistas por pesquisadores, como um avanço tecnológico de importância para a conservação do solo e para a produção de alimentos. No Brasil, essa técnica SPD foi utilizada por produtores do estado do Paraná, que foram os primeiros a adequar essa técnica agrícola as lavouras de clima tropical e subtropical (Motter e Almeida, 2015).

No Brasil cerca de 7,8% de sua área territorial é utilizada para produção agrícola, o que corresponde a aproximadamente 66 milhões de hectares. Deste montante, aproximadamente, 33 milhões de hectares utilizam o sistema de plantio direto (Salton et al., 1998).

O SPD, mostrado nas figuras 1 e 2, fundamenta-se em três pilares: (i) plantio sem revolvimento do solo e restrito à linha de semeadura; (ii) cobertura permanente do solo, com plantas vivas ou mortas ou palhadas; e (iii) diversificação de plantas na rotação de cultivos (Sá, 2019). Trata-se de uma técnica que traz benefícios dentro e sobre o solo.

Entende-se que cobertura viva são plantas que atuam como cobertura do solo, uma vez que são mantidas com suas raízes e as plantas em superfície. Cobertura morta são plantas que são roçadas e utilizadas para cobrir o solo como matéria orgânica morta. Estas coberturas geram benefícios químicos, físicos e biológicos no ambiente, como acúmulo de matéria orgânica, aumento da capacidade de retenção de água, maior disponibilidade de micro e macro nutrientes e melhoria da estrutura do ambiente como um todo (Espíndola, 1997).

Desta forma, é possível observar redução dos custos de produção, uma vez que se utilizam menos operações agrícolas necessárias no manejo das áreas, menos operações de preparo para o plantio, resultando em economia na utilização e menos aquisição de máquinas agrícolas, economia de combustível e custos de mão de obra (Fuentes-Llanillo et al., 2021).

Figura 1. Sistema de plantio direto (SPD). Solo com palhada de aveia, pronto para receber o plantio de soja. (Foto: Eliana Batista, 2022)



Figura 2. Sistema de plantio direto (SPD). Plantação de soja sobre palhada de aveia. (Foto: Eliana Batista, 2022)



O SPD é uma técnica agrícola sustentável, reconhecida mundialmente. Desde o início de sua implantação, observa-se uma redução dos impactos negativos ao solo, como a redução da emissão de gases poluentes. Esse sistema possibilita a manutenção da qualidade do solo para as culturas sucessivas, bem como existe um ajuste para qualquer bioma (Motter e Almeida, 2015).

Trata-se de um sistema de semeadura com aplicação de sementes e adubo sobre o solo não revolvido, utilizando-se máquinas específicas. Com a abertura de um sulco, com profundidade e largura necessárias para garantir uma boa cobertura e contato da semente com o solo. Este sistema de plantio prepara o solo no máximo 25% a 30% de sua superfície, o que pode favorecer o desenvolvimento e a sobrevivência de algumas pragas. O controle de plantas daninhas, operação fundamental no sistema, é geralmente feito com herbicidas aplicados antes ou depois da instalação da cultura (Trevisan e Rodrigues, 1985).

O SPD contribui com o aporte de resíduos vegetais, ou seja, da palhada, a qual fica na superfície do solo e o protege da radiação solar, da ação da chuva, auxilia no controle da erosão e contribui para a preservação de sua estrutura física, química e biológica. Além disso, favorece o manejo integrado de pragas, de plantas daninhas e doenças, utilizando menores doses de defensivos e fertilizantes (Embrapa, 2019).

## 2.4 ROTAÇÃO DE CULTURAS

A rotação de culturas adota plantios diversificados de espécies agrícolas como plantas de cobertura, principalmente com associação de leguminosas e gramíneas, as quais possuem sistemas de raízes diferentes entre si. Cada espécie agrega ao solo, ao ambiente e para a cultura sucessora uma ação residual positiva (Salton et al., 1998).

A rotação favorece o aumento dos teores de matéria orgânica e nitrogênio, colabora com a reposição de nutrientes e contribui para reduzir a amplitude das variações das temperaturas e umidade no solo, além de favorecer a atividade biológica (Embrapa, 2019). Esta prática promove melhoria na fertilidade das áreas cultivadas e na produtividade das culturas implantadas no local.

Exemplos da utilização da rotação de culturas pode ser observada nas figuras 3 e 4, em que em 2021 havia a plantação de milho e no ano seguinte (2022) a plantação de soja, no mesmo local. Na entressafra foi cultivado plantas de cobertura, aveia preta (*avena strigose*), para obtenção de palhada, na mesma área de 100 hectares, na região sudoeste do estado de São Paulo.

Figura 3. Plantação de milho em 2021. (Foto: Eliana Batista, 2021)



Figura 4. Plantação de soja em 2022. (Foto: Eliana Batista, 2022)



Dentre os vários benefícios obtidos ao se implantar o sistema de rotação de culturas, pode-se mencionar, que este colabora para a diminuição da acidez do solo. Com o aumento do pH para próximo de 6,0, este colabora com a disponibilidade de nutrientes que poderão ser absorvidos pelas plantas (Roscoe et al., 2002).

Com o passar dos anos, os cultivos anuais e contínuos no mesmo local, como acontece em áreas irrigadas por pivô central, apresentam queda na produtividade.

Esta queda de produtividade acontece pois se alteram as características do solo e as condições do ambiente se tornam propícias à multiplicação de doenças e pragas. Uma forma de eliminar ou atenuar esses problemas é a prática da rotação de culturas (Silveira et al., 1994).

A presença de espécies diversificadas dentro de um sistema agrícola de rotação de culturas deve observar as condições ambientais de cada região. Há plantas que se adaptam a regiões com temperaturas mais baixas e outras se adaptam a regiões com temperaturas mais altas ou amenas. Dentre as variedades de espécies de plantas de cobertura, pode-se citar: aveia (*Avena strigosa*); milho (*Pennisetum glaucum*); várias espécies de pastagens, como brachiária (*Urochloa brizantha*; *Urochloa decumbens*; *Urochloa ruziziensis*); guandu (*Cajanus cajan*); tremoço (*Lupinus*), cultivadas em diferentes épocas e regiões do país (Salton et al., 1998).

Dentre os vários benefícios que essas e outras plantas que participam da rotação de culturas trazem, destacam-se a contribuição para aumento do carbono nos solos e da fertilidade para o desenvolvimento das plantas dentro e fora do solo, colaboram com a preservação do ambiente agrícola e para o aumento da produtividade e rentabilidade dos agricultores. Observa-se ainda a diminuição de perdas por lixiviação de nutrientes solúveis na forma de nitrato, também, minimizam problemas de infestação de insetos, de pragas, de plantas daninhas e de doenças.

Para que este processo de rotação de culturas seja bem-sucedido é fundamental a implantação do plantio direto, simultaneamente, com o objetivo de produção de palha e resíduos de plantas no complexo da rotação (Salton et al., 1998).

## 2.5 ADUBAÇÃO VERDE, PLANTAS DE COBERTURA E MATÉRIA ORGÂNICA NO SOLO

A adubação verde é uma prática que possibilita a conservação do solo, conhecida desde a era cristã por recuperar solos degradados pelo cultivo, além de melhorar os que são naturalmente pobres, e preservar os que são produtivos. Nos anos 1960, a adubação verde foi perdendo importância com o surgimento de máquinas, equipamentos e insumos modernos. No entanto, a partir dos anos 1980, foi com o uso de adubação verde que a agricultura deu um salto de qualidade (Carlos et al., 2014).

Os adubos verdes tornaram-se componentes fundamentais em arranjos de sucessão e rotação de culturas, que viabilizaram tanto o SPD, quanto a integração lavoura-pecuária e os sistemas agroecológicos de produção (Carlos et al., 2014).

A adubação verde consiste no cultivo de plantas nas entressafras, no mesmo local de plantio das culturas anuais ou principais, para contribuir com a reposição dos

nutrientes exportados do solo, como mostra a figura 5, plantação de aveia fazendo esta função no solo. Na maioria das vezes, com a intenção de semear sem colher, para gerar massa verde.

As plantas de cobertura, possuem características recuperadoras, recicladoras, protetoras, melhoradoras e condicionadoras de solo (Rossi et al., 2014).

Figura 5. Plantação de aveia, após colheita do milho. A aveia foi utilizada como planta de cobertura - adubação verde (Foto: Eliana Batista, 2022).



Culturas e resíduos orgânicos apresentam diferentes relações carbono – nitrogênio (C/N), ou relação C/N. Na tabela 2, é possível observar a relação C/N de algumas culturas. Quanto mais alta esta relação, maior o tempo de permanência no solo. A relação C/N de uma cultura está diretamente relacionada com a decomposição e a mineralização.

Alta razão C/N indica menores taxas de decomposição e seus resíduos permanecem por mais tempo no solo e, do contrário, baixa razão C/N indica rápida decomposição, o material é mineralizado tornando a disponibilidade de nutrientes no solo mais rápida, suas palhadas ou permanências no solo somem rapidamente (Embrapa, 2015). Uma razão C/N média refere-se a um valor médio de cerca de 24 (Torres et al., 2005).

Tabela 2. Valores aproximados da relação carbono – nitrogênio (C/N) de algumas culturas.

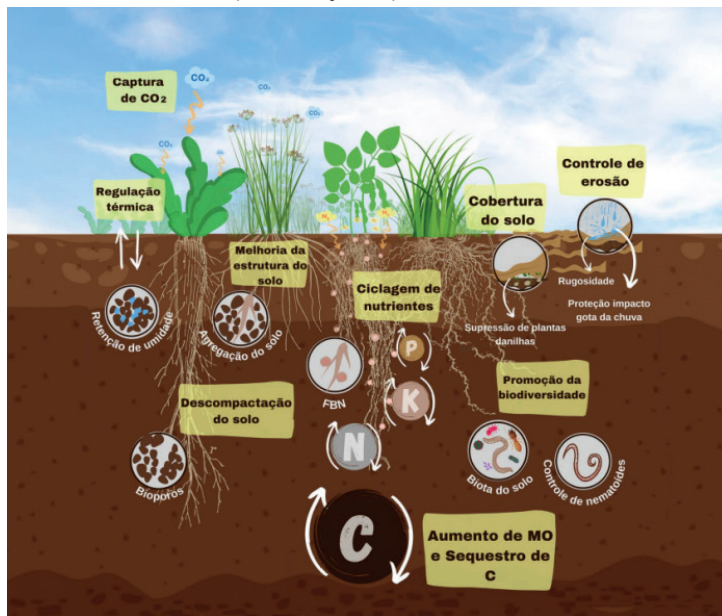
Culturas	Relação Carbono - Nitrogênio (C/N)
Crotalária	18,1
Milheto	25,3
Feijão guandu	16,6
Aveia	28,3
Braquiária	19,6
Trigo	80
Milho	57

Fonte: Torres et al. (2005)

Tem-se como algumas plantas de cobertura: crotalaria (*Crotalaria breviflora*; *Crotalaria ochroleuca*; *Crotalaria juncea*; *Crotalaria spectabilis*), milheto (*Pennisetum glaucum*), feijão guandu anão (*Cajanus cajan*), aveia preta (*Avena strigosa*), trigo (*Triticum spp.*), amendoim forrageiro (*Arachis pintoi*) (Cherubin, 2022). A utilização de plantas de cobertura nos solos agricultáveis, trazem benefícios que estão além de cobrir a sua superfície. Normalmente, elas serão dessecadas, roçadas e mantidas como palhada no solo.

Na figura 6 é possível observar um esquema que mostra os múltiplos benefícios e as melhorias na saúde do solo, bem como da melhoria dos componentes físicos, químicos e biológicos que são os responsáveis pelo funcionamento de ecossistemas vivos. As plantas de cobertura promovem o controle da erosão através da redução dos impactos das chuvas, melhor controle da enxurrada, e melhoria da resistência do solo à desagregação (Vitti et al., 2015).

Figura 6. Benefícios fornecidos pela utilização de plantas de cobertura (Foto: Cherubin, 2022).



As plantas que formam a adubação verde auxiliam na regulação térmica do solo, na redução da evaporação e no controle de plantas daninhas, seja competindo por luz, água e nutrientes, efeito alelopático, ou atuando como barreira física contra a emergência de plantas invasoras (Cherubin, 2022).

O uso das plantas com a intenção de manejar adubação verde são as chaves para aumentar a qualidade do solo. Além do seu baixo custo, verifica-se o potencial de

umentar a produtividade das culturas comerciais em rotação, com ganhos que podem chegara 20%, na melhoria da saúde do solo (química, física e biológica), e no aumento das taxas de sequestro de carbono nos solos agrícolas (Cherubin, 2022).

Raij (1969) verificou que todos estes benefícios são possíveis de serem obtidos devido a presença da matéria orgânica do solo (MOS), a qual participa e colabora para o aumento da capacidade de troca de cátions (CTC), que está relacionada à fertilidade dos solos. Portanto, entende-se que há uma correlação entre a capacidade de troca de cátions (CTC), a matéria orgânica, e o pH dos solos, pois a adubação verde colabora para que se chegue a estes níveis no ambiente, em que o pH que deve estar acima de 5,5.

Na tabela 3 é possível observar a contribuição da MOS no solo para aumento da CTC, em solos de diferentes classes no território brasileiro.

Tabela 3. Contribuição da matéria orgânica no solo para aumento da capacidade de troca de cátions (CTC).

Região (R)/Estados (E)	Nº de classes de solos avaliadas	% da CTC devido a presença da matéria orgânica do solo	Referências
R. Cerrado	14	75 à 85	(Resck, 1998)
E. Paraná	12	75 à 90	(Pavan, Bingham e Pratt, 1986)
E. São Paulo	16	70 à 74	(Raij, 1969)

A matéria orgânica presente no solo exerce papel relevante quanto aos aspectos físico, químico e biológico. A fonte desta matéria orgânica, a sua composição, as suas alterações e suas funções, vêm sendo estudadas em muitas pesquisas ao redor do mundo, ainda com amplas áreas a serem para exploração, em diferentes aplicações na ciência do solo (Soares et al., 2009), além da sua importância no fornecimento de nutrientes, na de retenção de água, e em melhorias significativas no ambiente para o desenvolvimento das plantas em todos os seus estádios e outros fatores.

### 3 CONSIDERAÇÕES FINAIS

Avaliar e monitorar a qualidade do solo é fundamental para: (i) manter os agroecossistemas funcionando; (ii) para traçar estratégias de produção e auxiliar nas tomadas de decisões e; (iii) para sustentabilidade dos ambientes produtivos. As práticas conservacionistas como SPD, rotação de culturas e adubação verde são relevantes, trazem benefícios e devem ser largamente utilizadas, principalmente ao se buscar uma agricultura sustentável.

Vale lembrar que, uma vez que se busca a preservação do solo, as camadas férteis serão preservadas e os principais fatores que influenciam a dinâmica de nutrientes do mesmo. O manejo adequado colabora com a conservação física, química e biológica que resulta nesta fertilidade tão necessária, sendo esta uma ferramenta fundamental para a obtenção de boa produtividade.

Diversos estudos apresentam que os métodos conservacionistas buscam menor mobilização juntamente com a manutenção de resíduos vegetais na superfície do solo, seja cobertura viva ou morta. Com a redução desta mobilização se atenuam os impactos negativos, como a degradação de sua estrutura. Um ponto a ser observado em uma área agrícola é a vulnerabilidade dos solos, pois mediante isso o agricultor poderá saber quais serão as limitações dos mesmos, e buscar formas de resolver os problemas.

Por mais que se apliquem técnicas de manejo preservando o solo, as tecnologias das máquinas agrícolas estão distantes de apresentarem equipamentos mais leves que causem menor impacto, e dessa forma, muito ainda precisa ser feito para preservação dos agroecossistemas.

De modo geral, observa-se que existem inúmeros desafios a serem vencidos, para que se alcance o manejo do solo de forma ideal e sustentável em curto, médio e longo prazos. Desafios que seguem além da conservação do solo, como: (i) altos custos na obtenção de equipamentos, de insumos para produção, seja em pequena ou larga escala, (ii) é comum observar a falta de incentivos para melhorias de preços de comercialização de *commodities* ou produtos do setor primário que, muitas vezes, geram baixos retornos financeiros, (iii) falta de conhecimento e informações sobre como, quando, onde, o que e em qual quantidade utilizar insumos agrícolas que colaboram para a fertilidade do solo e, também, como maneja-lo de forma a preservar o ambiente.

Existem inúmeras tecnologias da ciência do solo que proporcionam as áreas agricultáveis aumentos significativos de seu potencial produtivo, porém os indivíduos que o manejam necessitam ter mais acessos a estas importantes informações. Uma saída poderia ser incentivos aliados a programas didáticos, levando acessibilidades de conhecimentos sobre manejos as áreas rurais, onde estão os agricultores e pecuaristas, (iv) altos custos e falta de investimentos governamentais nos transportes de mercadorias, até que os produtos cheguem ao consumidor final. Estes altos custos de transportes geram aumentos de preços em toda a cadeia do agronegócio e diminuem lucros, a começar a jusante, com os produtores rurais.

Ao se conscientizar da necessidade das ações conservacionistas e aplica-las, haverá uma contribuição para atenuar as perturbações antrópicas, no entanto, elas devem ser adotadas em conjunto com outras formas de manejo para melhores resultados.



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# CAPÍTULO 2

## PROS AND CONS OF USING FORESTRY AS A COMPENSATION MECHANISM FOR GREENHOUSE GASES EMISSIONS ON NEW ZEALAND PASTORAL FARMS

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**ABSTRACT:** Mitigating or offsetting GHG emissions from pastoral farms is a complex issue. Within New Zealand, mitigation options include fine-tuning farm systems to make them more efficient, and/or land use change into horticulture or arable cropping. Both these approaches have limitations to the degree to which GHG emissions can be reduced. Offsetting via forestry is often the cheapest and most practical option for many farms, although the system to achieve this is also relatively complex and does not reduce gross emissions. The introduction of a value for carbon has resulted in carbon farming being a much more profitable option compared to sheep & beef farming, which has wider economic implications. The proposed introduction of a farm-level levy also provides an incentive to use forestry to offset this.

**KEYWORDS:** Greenhouse gas mitigation. Agricultural systems. Carbon farming. Forestry offsets.

### 1 BACKGROUND

Within New Zealand there are a range of factors driving the country's response to climate change. These include:

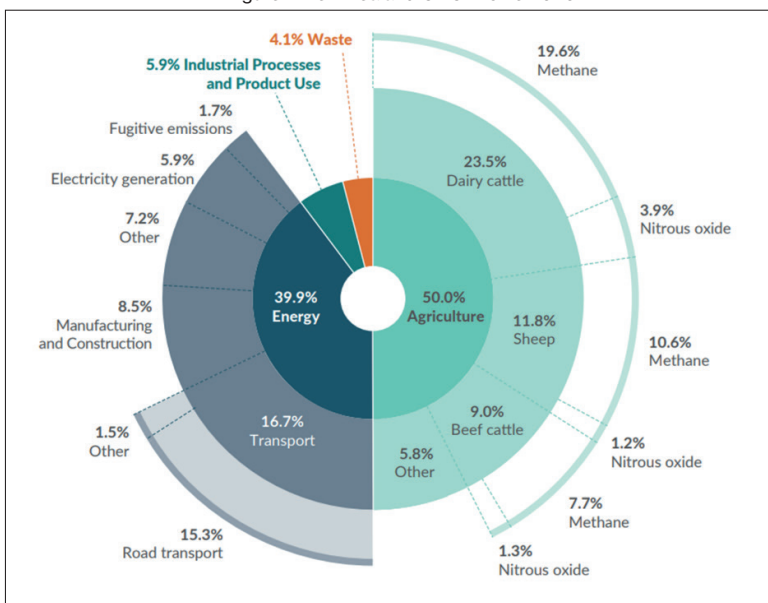
- Signatory to the United Nations Paris Agreement (2020-2030), where our Nationally Determined Contribution is a reduction in emissions of 50% below 2005 levels by 2050.
- Domestically, the Climate Change Response (Zero Carbon) Amendment Act was passed in 2019, with three key factors:
  - Carbon dioxide and nitrous oxide ("long-lived gases") are net zero by 2050
  - Methane reduced to 10% below 2017 levels by 2030, and to 24-47% below 2017 levels by 2050
  - Government must have an Emissions Reduction Plan and a National Adaptation Plan.

As can be seen by the reduction targets, New Zealand has split reduction targets between long-lived gases ( $\text{CO}_2$ ,  $\text{N}_2\text{O}$ ) and  $\text{CH}_4$ , which is a relatively short-term gas. Currently it is the only country in the world to do this.

- The Emissions Trading Scheme (ETS) was set up in 2008, as the main mechanism to value carbon within the domestic economy. People sequestering carbon (very largely by growing forests) are granted carbon credits (NZ Units, = 1 tonne  $\text{CO}_2$  equivalent) by the government, and then in turn sell these to greenhouse gas emitters, who then have to surrender the NZU's back to government. All sectors of the New Zealand economy, including agricultural processors, are included within the ETS, but not farming.
- In 2019 the government set up a working group which included all the agricultural organisations, representatives of Māori farming, and government to develop a farm-level pricing system for agricultural emissions as an alternative to the ETS. This group, known as *He Waka Eke Noa* (We're all in this together) recommended that a farm-level pricing mechanism be set up, with differential pricing for  $\text{CH}_4$  and  $\text{N}_2\text{O}$ . The intent is that farmers will face this pricing mechanism from 2025.
- In 2018 the Climate Change Commission was set up, initially as an "interim" commission, which is an independent body formed to provide advice to government on climate change matters, including emissions budgets.

The key issue that faces New Zealand is that our greenhouse gas (GHG) emission profile is very different to other developed countries, where emissions from our agricultural sector make up 50% of our gross emissions. The nearest OECD country is Ireland, whose agricultural GHG emissions make up 37% of their total emissions, while for most OECD countries, agriculture makes up 5-10% of gross emissions. For New Zealand this is important, as pastoral agriculture makes up around 65% of New Zealand's mercantile exports.

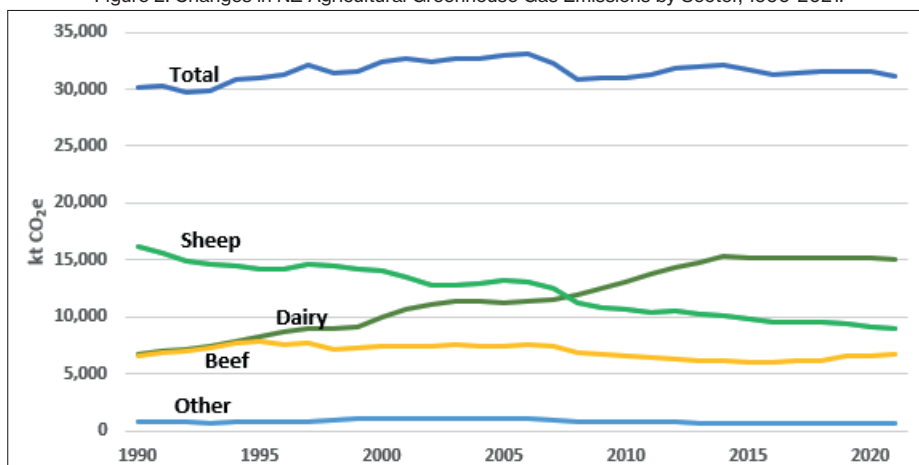
Figure 1: New Zealand GHG Profile 2020.



Source: NZ GHG Inventory 1990-2020, published April 2022 <https://environment.govt.nz/publications/new-zealands-greenhouse-gas-inventory-1990-2020/>

As can be seen from Figure 1, dairying makes up 47% of agricultural emissions, with sheep & beef farming making up 42%. Within the energy sector, approximately 85% of New Zealand electricity generation is renewable (largely hydro-electric) and moving up to around 95%. This means that the two main sectors where GHG reductions can be made is agriculture and transport.

Figure 2: Changes in NZ Agricultural Greenhouse Gas Emissions by Sector, 1990-2021.



Source: MfE 2022.

Figure 2 shows a reduction in GHG emissions from the sheep sector, due to a large reduction in stock numbers since 2019, a combination of reduced numbers on-farm, plus land use change into either dairying or forestry. Dairy emissions have increased because of significant land use change into dairying over the period, driven by its superior profitability. Beef emissions have been relatively static.

Over the period 1990 to 2020, total livestock GHG emissions ( $\text{CH}_4 + \text{N}_2\text{O}$ ) have increased by 16.6%, while  $\text{CH}_4$  emissions have increased by 8.4% and  $\text{N}_2\text{O}$  emissions by 49.4% (driven largely by significant increases in nitrogen fertiliser usage over the period). (MfE 2022).

## 2 HE WAKA EKE NOA

As noted, this group, comprising agricultural bodies, Iwi, and government, was set up in 2019 to determine a GHG emissions pricing mechanism for farming which would operate outside of the ETS. The overall system agreed by government included:

- An on-farm pricing system, where the price of  $\text{CH}_4$  would be set by Government, while the price of  $\text{N}_2\text{O}$  would be linked to the ETS price (5% in 2025, rising by 1% per year through to 2030 when it would be revised).
- A centralised and standardised GHG calculator to estimate annual GHG emissions at the farm level.
- Emissions levy to be set at the lowest price possible to achieve outcomes; revenue used to incentivise behaviour change.
- Incentive payments to make uptake of mitigation technologies and practices more cost-effective.
- An expansion of the definition of “forestry” to allow for additional areas to be included for carbon sequestration, and for these to eventually be included within the ETS.
- Farms which would face an emissions price would be;
  - Greater than 550 stock units (sheep, cattle, deer), or
  - Greater than 50 dairy cows, or
  - Apply more than 40 tonnes of nitrogen through synthetic fertiliser.

## 3 EMISSIONS TRADING SCHEME

The ETS as mentioned is the main market mechanism for valuing carbon in New Zealand, whereby GHG emitters can buy such carbon credits as a means of offsetting their emissions. The main means of sequestering carbon is via forestry, and there are a number of rules around eligibility of forests to be registered in the ETS:

- Must have been planted, or regenerated, after 1 January 1990
- Must be 1 hectare or larger,
- Forest canopy width must be greater than 30 metres on average,
- Trees must be able to grow 5 metres or taller,
- The tree canopy must cover at least 30% of the ground, as measured on the horizontal plane.

It excludes any forest which existed prior to 1 January 1990, horticultural tree crops, and shelter belts.

In 2020 the government introduced some further refinements to the ETS:

- A cap on total emissions that declines over time, in line with emissions budgets (as set by the Climate Change Commission)
- Auctioning, which was introduced in 2021, whereby the government auctions off a set amount of NZU's quarterly (usually around 4.5 million), as a means of raising funds for Climate Change related actions. Note these NZUs are not backed by any sequestration.
- A phaseout of the free allocation to industrial emitters. This free allocation was 95% in 2020, and reduces by 1% per year through the 2020's, 2% per year through the 2030's, and 3% per year through the 2040's.
- A floor price for NZU's - \$30<sup>1</sup> in 2022, rising to \$40 in 2027 and increases by 2% per year.
- A ceiling price for NZU's - \$70 in 2022, rising to \$129.97 in 2027, and again increasing at 2% per year. The Climate Change Commission has been recommending an increase in the ceiling price to \$171 immediately, in order to accelerate GHG mitigations.

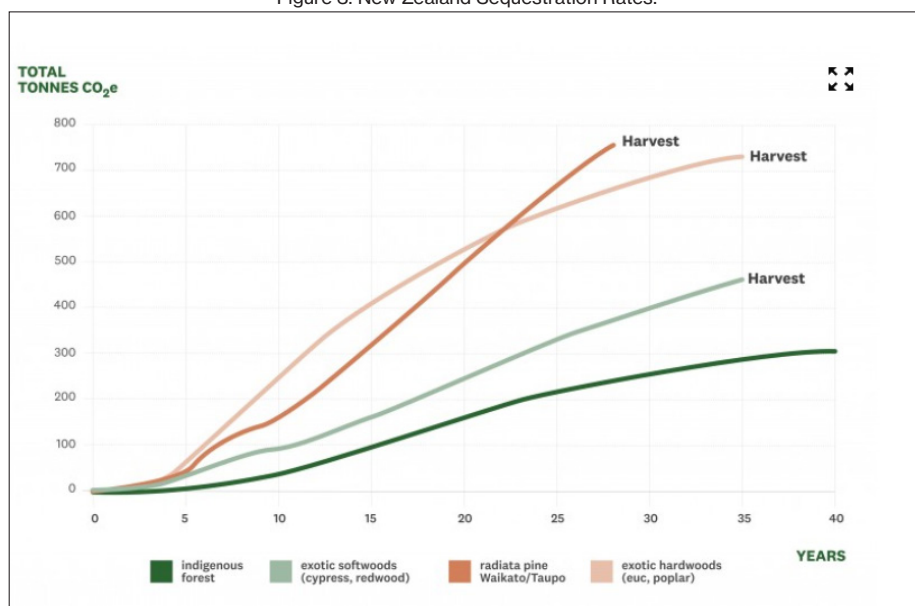
The amount of sequestration claimable is also controlled to a degree. Any forests less than 100ha in size, in aggregate, are required to use the sequestration rates as per the Ministry for Primary Industry (MPI) Look-up tables (MPI 2017), whereas forests 100 hectares or greater need to be physically measured every 5 years to determine the actual sequestration rate.

The government has stated that if it is not possible to enact the He Waka Eke Noa provisions, farming would be placed within the ETS.

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<sup>1</sup> All prices are in \$NZ

Figure 3: New Zealand Sequestration Rates.



Source: MPI Carbon Look-up Tables for Forestry.

As can be seen from Figure 3, the fastest sequestration rates are from radiata pine, the main production species grown in New Zealand (90% of production forests), averaging up to 25 tonnes CO<sub>2</sub>e per hectare per year, while indigenous forest average around 6.5 tonnes CO<sub>2</sub>e per hectare.

In January 2023, the regime whereby carbon credits could be claimed from forestry was simplified down to two mandatory options:

- (i) Averaging. At the start of the ETS, forest owners could claim carbon credits throughout the life of the forest, but needed to repay these, at the price on the day, when the forest was harvested. When the forest was replanted, further carbon credits could be claimed, until again when the forest was harvested. This regime is called the “stock management” regime.

From 1 January 2023, all production forests planted and registered for the ETS must go into the Averaging scheme. Under this system, a forest owner can claim roughly half the carbon sequestered in the first rotation and does not need to pay this back at harvest, provided the forest is replanted. Carbon is claimable only in the first rotation, with none thereafter.

The government has set the period under which carbon can be claimed under the averaging scheme, relative to average rotation lengths:



- Pines - 16 years
  - Exotic softwoods (e.g. cypress, redwood) - 22 years
  - Exotic hardwoods (e.g. eucalypts, poplars) - 12 years
  - Douglas Fir - 26 years
- (ii) Permanent forest. As the name suggests, this is a permanent forest which will never be harvested, and where the carbon sequestration claimable is under the stock management approach – i.e. you can claim carbon for as long as the forest is growing. If the forest is ever harvested, or leaves the ETS, then any carbon credits claimed must be repaid. This regime does allow for selective logging, provided the key aspects of the ETS are retained, e.g. 30% canopy cover.

#### 4 FARM LEVEL GHG EMISSIONS

The key determinants of GHG emissions at a farm level are:

- The amount of dry matter (DM) consumed by the animals. There is a direct correlation with the amount of methane produced, and a strong correlation with nitrous oxide emissions, which is then also heavily influenced by;
- The amount of protein in the diet. Protein level in New Zealand pastures are generally quite high – well above average ruminant requirements.
- The amount of nitrogen fertiliser used. While there are some direct  $N_2O$  and  $CO_2$  emissions when nitrogen fertiliser is applied to the soil, the key reason for most New Zealand farmers using nitrogen fertiliser is to grow more pasture – i.e. increase the amount of DM on offer to the animals.

With respect to mitigating farm-level GHG emissions therefore, the need is to reduce all of the above, especially DM consumed given it is the main driver by far. The split in the GHG on-farm emissions between  $CH_4$  and  $N_2O$  is roughly 80:20.

Table 1: Average New Zealand Farm-Level Biological GHG Emissions

	Average T $CO_2e/ha$	Range T $CO_2e/ha$
Dairy	9.6	3.1-18.8
Sheep & Beef	3.6	0.12-7.1

Source: Dairy NZ 2017, AgResearch 2020.

Table 1 shows the per hectare GHG emissions, with a wide range within each sector, driven largely by the intensity of the farming system, and the level of any feed

inputs. The proposed carbon pricing levy to be introduced in 2025 will be based on the total farm emissions, which has implications for the sheep & beef sector.

Table 2: Total Farm Emissions and Profitability.

	Average area (ha)	Average T CO <sub>2</sub> e/ha	Total T CO <sub>2</sub> e	5-year Average EBITDA/ha*
Dairy	155	9.6	1,488	\$3,418
Sheep & Beef	695	3.6	2,502	\$371

\*Dairy NZ Economic Survey 2020/21, Beef + Lamb NZ Economic Survey 2020/21.

Table 2 shows that while the per hectare emissions from sheep & beef farms are 37.5% of that for dairying, total emissions are 68% higher due to the much greater area of the average sheep & beef farm. Which means they will be facing, proportionally, a higher emissions levy, while total profitability is around 50% of that of the average dairy farm. Hence their greater vulnerability to the emissions levy.

One of the options for farmers to mitigate GHG emissions is to alter or fine-tune their farming systems. Research by Journeaux and Kingi 2020, and Journeaux et al 2022(a), has shown that such fine tuning is possible, with reductions in GHG emissions on pastoral farms in the order of 2-10%. Much of this is based around reducing stocking rates, while at the same time increasing per animal productivity in order to maintain or improve farm profitability. Other options include finishing animals to target weights more quickly, or reducing exogenous inputs (e.g. supplementary feed and/or nitrogen fertiliser). The impact of the latter options varies depending on the degree of such inputs, and in almost all cases, reduces farm profitability.

Caution is also needed around such farm-system options, as they require adaptations in farm management to ensure they are successful. Reducing stocking rates means that grazing management must improve. Otherwise, pasture quality is lost, resulting in (often) significant reductions in production and hence profitability. Other options require changes in farm management which will take several years to achieve; there are no overnight fixes.

## 5 FORESTRY AS AN OFFSET

The decision whether to mitigate or offset an environmental externality depends largely on two factors; the practicality of either and what is the least-cost option.

Within New Zealand, the use of forestry sequestration to offset on-farm emissions is readily accepted, especially given the relative practicality and cost effectiveness of doing so. In noting this, there are of course several issues to take into account.

Table 3: Area required (ha) to offset on-farm emissions.

% offset:	5%	10%	25%	50%	100%
155 ha dairy farm	3.7	7.4	18.5	37.2	74.4
695 ha sheep & beef farm	6.3	12.5	31.3	62.6	125.1

This shows the area (in hectares) need to be planted in *pinus radiata* to offset a percentage of the farm emissions, relative to the national average farm sizes. At the bottom end of the scale, i.e. 5% offset, finding 3.7 hectares on a 155 hectare dairy farm would be something of a push, whereas finding 6.3 hectares on a 695 hectare sheep & beef farm would be very straightforward. At the other extreme, planting 74.4 hectares on a 155-hectare dairy farm means the dairy farm would largely cease to exist. Planting 125 hectares on a 695-hectare sheep & beef farm would be something of a push, given it equals 18% of the farm area – but not necessarily impossible.

Another key factor is that the forest need not be planted directly on the farm itself. As long as it was planted somewhere in New Zealand, the credits could be used for offsetting. Similarly, once an international trading system is developed, the trees could be planted anywhere in the world.

Using forestry as an offset is, in many cases, a least-cost option relative to other mitigation options. This can be illustrated by comparing relative shadow prices, calculated as change in farm profitability, divided by change in biological GHG emissions.

Table 4: Average Shadow Prices (\$/T CO<sub>2</sub>e)

	S&B	Dairy
Reduce stock numbers by 10%	\$91	\$562
Reduce stock numbers by 10% Increase Productivity	-\$348	-\$746
Plant 10% pines	-\$2	\$56
Plant 10% natives	\$180	\$225
50% N Fertiliser		\$145
No bought in supplement		\$90

Source: Journeaux and Kingi, 2022

Caution is needed in interpreting these prices, as they are averages based on a small sample. Nevertheless, they indicate the relative cost-effectiveness of using forestry as an offset.

In noting this, using forestry as an offset has its limitations, the main one being it is a relatively short-term solution. As noted earlier, under the averaging scheme, there is a limit on the time carbon credits can be claimed, and only in the first rotation. Assuming therefore that offsetting via forestry is the only mechanism to be used, then additional

areas need to be planted at the end of the averaging period. Under the permanent forest scheme, it is possible to plant an initial area which can cover the farm emissions for many years. This is illustrated below, given a hypothetical situation; assume 10 hectares of production pines is sufficient to offset the farm emissions. Under the averaging scheme, the carbon credits from this forest would last 16 years, which means a new area of 10 hectares needs to be planted every 16 years. Conversely, if a native forest was planted as a permanent forest, then planting 36 hectares in year 1 (the larger area is required due to the slower sequestration rate) would be sufficient.

Table 5: Illustration of using forestry as an offset.

	Production Pines (ha)	Total ha Pines	Total ha Natives
Year 1	10	10	36
Year 16	10	20	
Year 32	10	30	
Year 48	10	40	
Year 64	10	50	

This shows the key achilles heel of using production forestry as a carbon offset – that increasing areas need to be planted over time in order to maintain the level of offsetting.

While Table 5 would indicate the desirability of using a permanent forest such as native trees, and many farmers would prefer to plant natives, the biggest drawback is economics: establishing pines cost \$2,500-\$3,000/ha, which then sequester 20-25 Tonnes CO<sub>2</sub>e/ha/year, whereas natives cost anything from \$10,000- \$45,000/ha (median is \$13,000-\$15,000/ha) and sequester 6.5 tonnes CO<sub>2</sub>e/ha/year. The reason the establishment of natives is so much higher is due to several factors:

- Number of plants planted per hectare is much higher than pines; 2,000-3,000 stems/ha compared to ~1,000 stems/ha for pines. This is because they grow much slower and death rates are much higher.
- Because they grow slower, post-planting management (e.g. weed release) requirements are higher.
- Because there is limited stock available, cost per plant is higher.

## 6 CALCULATING THE VALUE OF OFFSETTING

At a national level, the calculation is straightforward, tonnes of gross emissions less gross sequestration equal net emissions. For example, in 2020 New Zealand's gross

emissions were 77,778 kilotonnes of CO<sub>2</sub>e, and sequestration was 23,313 kilotonnes CO<sub>2</sub>e, giving net emissions of 55,465 kilotonnes CO<sub>2</sub>e (MfE 2022).

At the farm-level, it is a bit more complicated. If a farm was seeking carbon neutral status, then the calculation would again be straightforward; tonnes of CO<sub>2</sub>e gross emissions less tonnes CO<sub>2</sub>e sequestered.

For the calculation of the farm levy however, everything is calculated through at a monetary value. The formula for this is: A + B – I – C, where:

A = price of methane (\$/kg CH<sub>4</sub>)

B = price of nitrous oxide (\$ T CO<sub>2</sub>e)

I = an incentive payment for using new technology, currently suggested as \$50/T CO<sub>2</sub>e reduced as a result of using the technology

C = the value of sequestered carbon (\$/T CO<sub>2</sub>e, aka the ETS price)

Table 6: Example of using forestry as an offset at the farm level.

	Total T CH <sub>4</sub> as CO <sub>2</sub> e	Total T N <sub>2</sub> O as CO <sub>2</sub> e	Assumed Sequestration (T CO <sub>2</sub> e)	Cost of CH <sub>4</sub>	Cost of N <sub>2</sub> O	Gross Levy	Value of forestry sequestration	Net Levy
<b>Dairy</b>	1,190	298	72	\$5,238	\$1,265	\$6,503	\$6,120	\$383
<b>Sheep &amp; Beef</b>	2,002	500	220	\$8,807	\$2,127	\$10,934	\$18,700	-\$7,766

Note:

- (i) The farms used are as per the national average farms (Table 2)
- (ii) The sequestration assumed is purely for illustration.
- (iii) The pricing used is that proposed by He Waka Eke Noa for 2025 (HWEN 2022)

As can be seen from Table 6, the value of the carbon credits from forestry has a significant impact when used to offset the carbon levy. This can also be illustrated using actual farms, where planting 10% of the farm into forestry has been modelled.

Table 7: Impact of Forestry Offsetting on Proposed Carbon Levy.

Sheep & Beef	Pastoral Area (ha)	Forest Area (ha)	Gross T CO <sub>2</sub> e Emissions	Gross Carbon Levy 2025	Forestry Credit	Net Levy 2025
<b>Base</b>	1,632		8,396	\$15,159		\$15,159
<b>Plant 10% forest - Pines</b>	1,470	162	7,329	\$12,092	\$304,317	-\$292,225
<b>Plant 10% forest - Cypress</b>	1,470	162	7,329	\$12,092	\$176,256	-\$164,164
<b>Plant 10% forest - Natives</b>	1,470	162	7,329	\$12,092	\$89,505	-\$77,413

Table 7 continued.

Dairy	Pastoral Area (ha)	Forest Area (ha)	Gross T CO <sub>2</sub> e Emissions	Gross Carbon Levy 2025	Forestry Credit	Net Levy 2025
Base	155.1		2,027	\$8,836		\$8,836
Plant 10% forest - Pines	140.1	15	1,806	\$7,112	\$26,520	-\$19,408
Plant 10% forest - Cypress	140.1	15	1,806	\$7,112	\$16,320	-\$9,208
Plant 10% forest - Natives	140.1	15	1,806	\$7,112	\$8,288	-\$1,176

Table 7 shows several things:

- Total gross emissions reduce as a result of the forestry planting, due to livestock being displaced by the trees.
- Selling the carbon credits as sequestered by the forestry significantly offsets the cost of the levy, realising that the income from the pines lasts 16 years, the cypress 22 years, and from the natives 200-300 years.
- The faster sequestration by the pines means that the credits generated are much more than from the cypress, which in turn is greater than from native trees.
- If, under the pine scenario, only sufficient carbon credits were sold annually to offset the levy, with the remainder held for later years, then the credits would be sufficient to offset the levy for 30+ years.

## 7 CARBON FARMING

The advent of the ETS and a value for carbon sequestration via forestry, has given rise to the practice of “carbon farming”, particularly in recent years as the carbon price has risen.

In this enterprise, farms are planted up into forestry, in most instances into *pinus radiata*, which tends to give the greatest returns, and following the end of carbon credits (under averaging), the forest then reverts to a production forest.

The driver for this is of course the economics of the system. As noted in Table 2, the 5-year average EBITDA for sheep & beef farming, is \$371 per hectare. Planted in pines, the same land would return around \$1,500 per hectare, for 16 years, and \$100-\$250 per hectare thereafter as a production forest. This means the internal rate of return from the carbon farming is 5-7 times that of sheep & beef farming.

This has driven investors, many from overseas, to buy up whole sheep & beef farms and convert them to forestry, and since 2017, an estimated 175,000 hectares of sheep & beef land has been sold for conversion<sup>2</sup>.

<sup>2</sup> Stuff, 15 November 2022. <https://www.stuff.co.nz/business/farming/130412293/12000-hectares-approved-for-sale-to-overseas-investors-for-forestry>

This in turn has created a degree of angst within New Zealand, particularly from farmers and rural communities whose livelihoods and social services are threatened.

This had led to calls within the community, including from the Parliamentary Commissioner for the Environment, and the Climate Change Commission, to limit the use of forestry as an offset, especially for industrial emitters/fossil fuel users, as they are currently very largely using forestry as an offset rather than mitigating emissions, as it's the least-cost option. It has also been pointed out that if New Zealand relies heavily on offsetting, then (a) our gross emissions won't reduce, and (b) eventually we'll run out of land to plant, aka Table 5.

A recent study by Journeaux et al (2022(b)), illustrates this. This analysis considered two sheep & beef farms, one in Northland, one in Hawke's Bay, which were progressively planted up into forestry: 10%, 30%, 100%, in pines, cypress, and natives. The impacts at both then farm level and regionally were then analysed. The results showed:

(i) At the farm level:

- Planting 10% of the farm into pines had no impact on the farm EBITDA, in the absence of a value for carbon. The areas planted were the lower productive areas on the farms, and therefore wider impact was minimal, given the returns for forestry were similar if not better than the farm returns on those areas.
- Planting greater than 10% of the farm into forestry progressively worsened the profitability of the farm.
- Introducing a value for carbon sequestration basically swamped the rest of the returns from the farming operation. The most profitable option was to plant 100% of the farm into pines, followed by 100% into cypress. All indigenous forest plantings resulted in a much lower EBITDA relative to the pastoral (base) operation.

(ii) At a regional level, this was analysed using Input/Output analysis:

- A positive impact in the initial year, for both value-add and employment, where the benefits of planting the forest offset the loss of the farming production.
- From then on, the impact was negative, both for the region in question and for the rest of New Zealand, through until the forest was harvested.
- In the year of harvest there was a significant increase in both value-add and employment as a result of the harvesting/processing. This resulted in an overall positive Net Present Value (NPV) for the

pinus scenarios, and the 10% Cypress scenario. All the indigenous scenarios had a negative NPV.

- From an employment perspective there is a necessity for planting and harvesting to be phased and sequenced in order to maintain a sufficiently skilled labour force for harvest. This was not undertaken in this analysis.
- The addition of a value for carbon provided no net gain in value-add. The impact of a value for carbon is essentially an internal wealth transfer, with no overall net benefit at a national level.

In many respects the mantra from this study is “forests on farms, not farms into forests”.

## 8 DISCUSSION

The advent of climate change policy, the development of the ETS and the fast-approaching pricing of agricultural emissions is a very complex area, and the implications of these are not well understood.

At a farm-level, there is some opportunity for farmers to reduce GHG emissions by fine-tuning farm systems and improving efficiency. Research would indicate this is relatively limited, to around 2-10% reduction while ensuring the farming system remains profitable. Achieving this will require improvements in farm management, and an extension programme to provide information and advice to farmers. This will take time, as there are no ready “quick fixes”.

Currently there are a number of potentially useful mitigation technologies being researched, but which will take some time to come to fruition and become commercially available.

The use of forestry therefore, as a means of offsetting, is likely to be a tool which many farmers are likely to use, particularly in the sheep & beef sector, and particularly as it is relatively practical, least cost, and has a significant impact in offsetting the proposed carbon levy.

The main drawbacks are that it is not a permanent solution, as more forestry areas would need to be planted in the future, and it does nothing to reduce gross emissions, which is what the regulations, and markets, are calling for. And if carbon farming continues whereby pastoral farms are planted up into forestry, it will radically, and adversely, change the character of rural communities and economies.

Note: New Zealand held a general election in October 2023, electing a new government. The previous government did not pass legislation in time to enact the He



Waka Eke Noa provisions, and the new Government has announced that they will delay the on-farm pricing mechanism potentially through to 2030.

## 9 DISCLOSURES

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## CAPÍTULO 3

### STRUCTURAL AND CHEMICAL CHARACTERISTICS OF WOOD GREENERY ORIGINATING FROM BOSNIA AND HERZEGOVINA

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**ABSTRACT:** The subject of research is wood greenery of beech (*Fagus sylvatica*), sessile oak (*Quercus petraea*), black pine (*Pinus nigra*) and Norway spruce (*Picea abies*) which

remains at cutting areas after regular felling in forests of Bosnia and Herzegovina. For each species were analyzed structural relations of main components: wooden twigs, bark that covers twigs and green leaves/needles. In addition were analyzed: crude nutrients, macro and micro elements, physiologically active matters and amino acids. The length of a basic twig is one of the features that characterizes wood greenery as raw material. The greatest absolute and average length has wood greenery of beech, followed by oak, spruce and then black pine. In a broader sense, wood greenery of deciduous trees is longer than that of conifers. Most leaves/needles in relation to the weight of the basic twig has spruce, then black pine, oak and beech. In deciduous trees, oscillations in chemical composition were observed in spring and summer. As a rule, the content of chemical elements and compounds takes higher values in spring compared to those in summer, observing that with vitamins, these differences are not large, while with carotene they are emphasized. In conifers, this regularity is less pronounced, when winter and spring are taken as reference seasons. Most parameters have higher values during spring, however, not a negligible number of those whose values are higher during the winter, such as carotene. The highest concentration of amino acids was recorded in oak, followed by beech. Significantly less amino acids were detected in wood greenery of spruce, and least in black pine. Individually, the most

abundant amino acid was glutamic acid in the wood greenery of the oak, followed by valine in the wood greenery of the beech. The least represented amino acid in all samples was methionine. From the aspect of production continuity, coniferous wood greenery is of somewhat greater importance because it is available throughout the year, and deciduous wood greenery only during the vegetation season. In practice, these differences can be mitigated by choosing the right schedule of felling operations.

**KEYWORDS:** Wood greenery. Structural characteristics. Chemical composition. Conifers. Deciduous trees.

## 1 INTRODUCTION

Every year in our forests the cut down is around 4.45 mil. m<sup>3</sup>, from which 3.70 mil. m<sup>3</sup> is of different forest assortments, which means the difference between gross and net mass is about 750,000 m<sup>3</sup>. Each year this biomass remains unexploited in our felling areas (Ljubojević, 2016). The thinnest portion of unused biomass makes so-called wood greenery (WG). It is also referred as „muka“, tree foliage, tree verdure, technical foliage and technical greenery (Keays, 1971; Tait *et al.*, 1982; Young, 1976). Three basic components of WG are: woody twigs, bark that covers them, and leaves or needles grown on them. The architecture of WG consists of one, more or less accentuated, main twig with lateral twigs. A twig consists of an axis with leaves attached in certain arrangement, including buds (terminal and axillary), lenticels, stipules and stipule scars. It is considered that the most physiologically active substances are found in green mass (leaves/needles), much less in bark and the least in twig wood. If twigs are younger, they still haven't gotten woody, so they contain a larger amount of physiologically active matters. With thicker branches, participation of undesirable ligno-cellulose component increases at the expense of physiologically active complex (Tomčuk *et Tomčuk*, 1966, 1973, Terzić, 1970; Terzić *et al.*, 1979).

The age of WG, primarily of its green components, has direct repercussions on the quality and quantity of raw materials and future products. Old leaves have to fight with younger leaves for water. They have a lower intensity of photosynthesis and lower nitrogen content per unit mass compared to younger or shorter leaves. However, plants with long-lived leaves are usually associated with nutrient-poor soils (Midgley *et Enright*, 2000). Unlike deciduous trees, where some species retain their leaves for only a few months to one year, conifers are known for their longevity. Pines retain needles for over three years and spruce for about five and a half years (Gower *et al.*, 1993; Midgley *et Enright*, 2000; Pensa *et Sellin*, 2002). These moments should also be taken into account when organizing regular production.

Early studies of chemical composition of conifers have shown that 1 kg of dry spruce needles contains up to 4,000 international units of vitamin C, up to 600 mg of

beta-carotene (provitamin A), between 920 - 2600 mg of flavonoids and up to 560 mg of vitamin E, in the form of alpha-tocopherol (Valdman, 1955). Subsequent research has shown that conifers also contain chlorophyll, free amino acids, fatty acids, sterols, including beta-sitosterol, sugars, macro- and micronutrients, as well as substances essential for animal nutrition (Kalinsh *et al.*, 1978). The same source states that WG of conifers also contains some undesirable ingredients, such as: tannins and resin acids, and a large amounts of lignin, which limit its use mainly to poultry and piglets. Research in USA has given affirmative judgments about the possibilities of feeding rabbits and small livestock with WG of poplar (*Populus spp.*) and black locust (*Robinia pseudoacacia*), provided that polyethylene glycol, or otherwise, cancels the negative effects of tannins (Ayers, 1992).

WG is a raw material for a larger number of products: vitamin-mineral flour (VMF), chlorophyll-carotene paste (CCP), vitamin concentrate,  $\beta$ -carotene, essential oils and their fractions, coniferous wax and balsamic paste, other products (cineole, sodium chlorophyllin, iodine phytalisin, camphor), (Ebele *et Kalnsh*, 1954; levins *et al.*, 1986; Solodky, 1947; Solodky *et Agranat*, 1971; Tomčuk *et Tomčuk*, 1966, 1973; Fisher, 1971). It is interesting to note that the first preparations from WG were consumed by humans and only then by animals. Namely, in the late 1930s, in the Kolyma prison within northern camps of Gulag system (USSR), *coniferous soup* was used as a prophylactic agent and remedy (Gorbatov, 1989). Somewhat later, in the same country, *coniferous water* was developed as a remedy for scurvy during the siege of Leningrad (today St. Petersburg) in World War II. Throughout 872 days of the siege, inhabitants of Leningrad called the *coniferous water* the *elixir of life*. And the third, by the volume of production, the most massive product from WG was created in the former Soviet Union. It is a vitamin-mineral flour (VMF), a preparation for feeding domestic animals. VMF is formed by short-term drying of WG at a high temperature without the presence of oxygen, and then by grinding it to the consistency of flour. The production and application of VMF began in the 1950s. With the production of VMF, agriculture was given a completely new product, also forestry found placement for large quantities of hitherto unused raw materials. In circles of scientific and professional public of that time, the value of VMF was considered to be equivalent to a grass meal (Kalinsh *et al.* 1978).

First industrial plant for production of VMF was built in 1956 in Latvia as part of Forestry Company „Volcano“ in area of Kuldīga. The applied technology was developed at the Latvian Academy of Sciences and the Latvian Academy of Agriculture under the leadership of Kalnsh and Abolinsh (Tomčuk *et Tomčuk*, 1966). The capacity of the plant was 45t of product per year. By the end of 1980s, the production of VMF in USSR

reached 190,000 tons per year (Daugavietis *et al.*, 2015). Parallel to the development of VMF, CCP production technology was developed at the Leningrad Forestry Academy under the leadership of Solodky. Unlike VMF, the use of CCP is not unambiguously determined. Tomčuk and Tomčuk (1966, 1973) quoted Solodky who writes that positive results were obtained in the treatment of the following human diseases: diseases due to A-hypovitaminosis, thermal and chemical burns, ulcers of various etiologies, eczema, *Trichomonas colpitis*, pseudo-erosion, folliculitis, boils, hydradenitis, trichophytosis, lichen planus, chronic atrophic rhinitis. Moreover, in animal diseases such as: cow endometritis, gastrointestinal diseases of calves and lambs, surface wounds and some skin diseases. However, Daugavietis *et al.* (2015) state that CCP was widely used as a feed ingredient in the amount of 0.3-0.4% from the animal feed base. CCP efficacy has been substantiated by extensive laboratory trials and in production conditions, referring to: Ebele *et Kalninch* (1954), Solodky *et Hinich* (1969), Fisher (1971).

Considering the number of plants built and volume of production in them (until 1970, there were over 250 plants for processing WG in USSR alone), it is quite logical that the structure and chemical composition of raw materials be regulated by local standards. However, the first standard (GOST 21769-76) was adopted only in 1976 (Anon., 1978). Among other things, it prescribed a thickness of basic branch of 8 mm, measured with the bark. Second standard (GOST 21769-84) was passed in 1984 (Anon., 1984). This standard refers to WG as a raw material for the production of VMF, as well as for freshly prepared food additives for domestic animals and poultry. The standard stipulates, among other things, that VMF must not be made from WG of: *Corylus* sp., *Cytisus* sp., *Daphne mezereum*, *Fagus* sp., *Juglans* sp., *Quercus* sp., *Rhamnus* sp., *Rhus* sp., *Sambucus nigra*. Depending on the content of leaves/needles, bark, wood, inorganic and organic impurities, WG was classified into three classes. Class I included raw materials in which the proportion of leaves/needles, buds and non-woody shoots is not less than 80% and the mass fraction of bark and twigs is not more than 15%. Class II included raw materials in which the share of the first component is not less than 70% and the second component is not more than 25%, while in class III these components took values above 60% and below 35%. For all three classes it was prescribed that the mass fraction of other organic impurities may not exceed 5%; nor that the mass fraction of inorganic impurities may exceed 0.2%.

The aim of this paper is to determine the morphological, structural and chemical characteristics of wood greenery originating from four widely distributed tree species in Bosnia and Herzegovina (B&H). The obtained results can usefully serve as one of the strongholds in the eventual decision-making on the production and processing of this, for the domestic conditions, new forest wood assortment.

## 2 MATERIAL AND METHODS

This research includes four species of trees, which inhabit significant areas of forests and forest lands in B&H. These are European beech (*Fagus sylvatica* L.), sessile oak (*Quercus petraea* (Matt.) Lieblein [Syn.: *Q. sessiliflora* Salisb.]), black pine (*Pinus nigra* Arnold) and Norway spruce (*Picea abies* (L.) Karsten [Syn. *P. excelsa* (Lam.) Link]).

In the introductory part, we quoted the Russian standard GOST 21769-84, which even prohibits the use of beech and oak WG for the production of VMF. The long-standing domestic livestock tradition, as well as numerous scientific and professional papers, affirm the use of WG of beech and oak, either as browse or as tree hay (tree fodder) in the years of crop failure (Bahtijarević, 1982; Ivanković *et al.*, 2005; Mackie, 1903; Nikolić, 1967; Rajkumar *et al.*, 2015; Topić *et Šupe*, 1996; Wessely, 1879). Therefore, we included these two species in our research.

Samples for the analysis were taken from three localities, situated in the northwestern part of the country. Beech and oak WG were taken from the high beech and oak forests on deep acid brown and ilimerised solils in Forest District “Gozna”, Forest Economic Area “Donjevrasko”. Spruce WG was taken from high spruce forest on deep acid brown and ilimerised solils in Forest District “Cvrcka”, Forest Economic Area “Čemerničko”. Black pine WG was taken from Forest enterprise “Industrial plantations” A.D., Banja Luka, Management unit “Kunova”.

The materials for the structural analysis of beech and oak WG were taken in the second week of May and in the second week of August 2018, while the material for the structural analysis of black pine and spruce WG, were taken in the second week of February and the second week of May 2018. Samples were taken from freshly felled trees by using portable electric pruning saws to separate branches 2-2.5 cm in diameter (thick to coarse branches). Afterwards, using a hand pruner and a caliper gauge, 10 mm thick twigs measured over bark, were separated; in older North American sources, a maximum WG twig thickness of 6 mm (0.24 inches), is recommended (Keays, 1971; Tait *et al.*, 1982). From each tree, 15 pieces of WG were taken, five pieces from the upper third of the canopy, five pieces from the middle of the canopy and five from the lower part of the canopy. In this way, an aggregate sample was formed with 45 pieces of WG of each species.

The structure of the basic twig of WG was analyzed using the sectional method, so that each twig was divided into five sections: the first section (I): twig thickness of 0.1-2.0 mm, II: 2.1-4.0 mm, III: 4.1-6.0 mm, IV: 6.1-8.0 mm, V: 8.1-10.0 mm. In each section, three basic components were carefully separated: the green part (leaves/needles), bark and wood, and weighed on a digital scale with a reading accuracy of 0.1 g.

The examination of chemical composition of WG was carried out in two phases. Crude nutrients, macro and micro elements and physiologically active matters were analyzed on the basis of material collected in 2018. The analysis of amino acids in WG of the investigated species was performed on the basis of material collected in spring of 2021, from the same locations as in 2018. In all samples, chemical analysis began no later than third day after collection, *i.e.* harvesting.

Individual chemical components were determined by classical methods of analysis. Total protein was determined by Kjeldahl. Fat determination was performed by the Soxhlet method. Crude fiber were determined according to Wende`s method. The ash was determined by burning the material at a temperature of 550 °C. Nitrogen-free extract (NFE) was determined as a supplement of up to 100% in relation to the total amounts of crude protein, fat, crude fiber and ash. The concentration of total chlorophyll and total carotenoids was determined by spectrophotometric measurements on a UV/VIS spectrophotometer and calculations according to the Lichtenthaler (1987) formulas. Vitamins C (ascorbic acid) and E were determined by reversed-phase HPLC on a C<sub>18</sub> column. Vitamin B<sub>2</sub> (riboflavin) was determined fluorimetrically and vitamin K using a fluorescence detector. The content of macro and microelements was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique. Analysis of amino acids contents were performed by L-8800 High-speed Amino Acid Analyzer (Hitachi), using appropriate syringe filters and standard amino acid solutions. The content of essential oil in the WG of black pine and spruce was determined using the Clevenger-type distillation apparatus, with flat-bottomed flask of 5,000 cm<sup>3</sup>. The weight of one batch was 300 g of raw material filled up with 3,000 cm<sup>3</sup> of water. The distillation lasted 4 hours. The amount of essential oil was read in cm<sup>3</sup>. In order to be able to determine the percentage oil content, the cubic oil content was multiplied by the specific weight of the oil, as follows: for black pine - 0.863 and for spruce 0.883 (Kapetanović *et al.*, 1988).

### 3 RESULTS AND DISCUSSION

The length of basic twig is one of many features that characterizes WG as a raw material. Results proved that greatest absolute and average length had WG of beech, followed by oak, spruce and then black pine (Tab. 1). In a broader sense, the WG of deciduous trees is longer than the WG of conifers (Fig. 1).

Table 1: The length of the basic twig of woody greenery.

Tree species	Average length of twig (cm)	Range (cm)	
		min	max
Beech	84.5	61	114
Oak	82.2	58	101
Black pine	39.9	21	50
Spruce	70.6	53	89

Two branch architectures predominate in beech: wider and narrower. In oak, the differentiation is more pronounced in the longitudinal than in the transverse view, so that we distinguish between longer and shorter branching. Black pine is also dominated by two types of arrangements: shorter and denser twigs and longer twigs with sparse needles. The main feature of spruce are laid and well-arranged twigs, which results in a large and dense coverage.

Figure 1: The appearance of wood greenery of beech (below) and black pine (above); (photo S. Ljubojević).



Mass structure of wood greenery by thickness of sections is shown in Table 2. Most leaves/needles in relation to the weight of basic twig has spruce - 76%, then black pine - 74%, oak - 50% and beech - 49%. Similar descending sequence was established by Terzić (1970) for the area of Maoča, in Krivaja river basin, in central B&H: spruce -78%, black pine - 74%, oak - 59% and beech - 55%. On average, spruce has the heaviest WG twig - 285.1 g, and the lightest beech - 125.9 g. On average, the heaviest WG (twig with Ø 10 mm at the thickest end) is discarded by spruce - 285.1 g, followed by WG of oak - 193.9 g, then WG of black pine - 170.8 g and finally of beech - 125.9 g.



An indicative parameter of structure of WG is a relative representation of WG components by thickness of sections (Tab. 3). In all observed species except spruce, with increasing of twig thickness, relative share of *green component* (leaf /needle) decreases. Also, in all observed species, the smallest reduction in the share of *green component* is between the fourth and fifth sections. This means that reducing the thickness of the basic twig from 10 mm to 8 mm does not contribute to a significant increase in the quality of WG. On the other hand, the inclusion of 8 -10 mm section in WG increases the degree of biomass utilization and reduces production costs. The most favourable structure of WG is found in spruce. With increasing thickness of the basic twig of spruce, there is no decrease in the relative share of the *green component*, but it even slightly increases at the expense of the other two components (bark and wood).

Table 2: Mass structure of wood greenery by thickness of sections and its components.

Thicknees of sections (mm)	Components of WG				Components of WG			
	Leaf/needle	Bark	Wood	Σ	Leaf/needle	Bark	Wood	Σ
	Beech (g)				Oak (g)			
0.1 - 2.0	16	0.2	0.3	2.1	2.8	0.5	0.3	3.6
2.1 - 4.0	6.3	1.5	2.0	9.8	9.0	3.0	2.2	14.2
4.1 - 6.0	12.6	4.4	7.0	24.0	18.0	6.3	6.2	30.5
6.1 - 8.0	18.0	6.9	13.9	38.8	28.4	17.2	17.2	62.8
8.1 - 10.0	23.4	9.7	18.1	51.2	39.8	20.6	22.4	82.8
Σ	61.9	22.7	41.3	125.9	98.0	47.6	48.3	193.9
%	49	18	33	100	50	25	25	100
	Black pine (g)				Spruce (g)			
0.1 - 2.0	-	-	-	-	-	-	-	-
2.1 - 4.0	12.4	2.1	0.7	15.2	18.1	3.8	2.6	24.5
4.1 - 6.0	27.7	5.1	3.0	35.8	52.0	11.0	7.8	70.8
6.1 - 8.0	37.5	8.2	6.8	52.5	64.0	9.3	8.0	81.3
8.1 - 10.0	49.2	9.0	9.1	67.3	83.2	13.3	12.0	108.5
Σ	126.8	24.4	19.6	170.8	217.3	37.4	30.4	285.1
%	74	14	12	100	76	13	11	100

Table 3: Relative representation of wood greenery components by thickness of sections.

Thickness of sections (mm)	Components of WG				Components of WG			
	Leaf/needle	Bark	Wood	Σ	Leaf/needle	Bark	Wood	Σ
	Beech (%)				Oak (%)			
0.1 – 2.0	76.0	9.0	15.0	100	77.8	13.9	8.3	100
0.1 – 4.0	66.4	14.3	19.3		66.3	19.7	14.0	
0.1 – 6.0	57.1	17.0	25.9		61.7	20.3	18.0	
0.1 – 8.0	51.5	17.4	31.1		52.4	24.3	23.3	
0.1 – 10.0	49.2	18.0	32.8		50.5	24.6	24.9	
	Black pine (%)				Spruce (%)			
0.1 – 2.0	-	-	-	100	-	-	-	100
0.1 – 4.0	81.6	13.8	4.6		73.9	15.5	10.6	
0.1 – 6.0	78.6	14.1	7.3		73.6	15.5	10.9	
0.1 – 8.0	75.0	14.9	10.1		75.2	13.8	11.0	
0.1 – 10.0	74.0	14.0	12.0		76.0	13.0	11.0	

The chemical composition of WG of beech, oak, black pine and spruce, originating from B&H, is shown in tables 4-7. Most proteins contain WG of oak during summer –56.4 g/kg<sub>FW</sub> and the least WG of black pine during spring - 37 g/kg<sub>FW</sub>. Conifers contain more than twice as much fat as deciduous trees. The least amount of moisture has WG of spruce during the winter, and the highest WG of black pine during the spring - 54.5%. It is interesting to note that a similar moisture content was registered in the WG of eastern white pine (*Pinus strobus*) in British Columbia – 53.2 % (Keays, 1971).

Table 4: Crude nutrients in beech, oak, black pine and spruce wood greenery originating from B&amp;H (values are based on fresh weight – FW.).

Components	Beech		Oak		Black pine		Spruce	
	Spring	Summer	Spring	Summer	Spring	Winter	Spring	Winter
Protein (g/kg <sub>FW</sub> )	44	49.7	49.2	56.4	31	37	38	34.6
Fat (g/kg <sub>FW</sub> )	16	14	16.5	15.9	54	56	49	54
Crude fiber (g/kg <sub>FW</sub> )	155	188	159	177	193.3	220.6	214	230.1
NFE (g/kg <sub>FW</sub> )	289.7	269.4	235.5	186.9	166.4	186	201.1	210
Ash (g/kg <sub>FW</sub> )	19.9	20.4	23.6	29.8	10.3	12.4	15.9	18.8
Dry matter (g/kg <sub>FW</sub> )	524.6	541.5	483.8	466	455	512	518	547.5
Moisture (%)	47.5	45.8	51.6	53.4	54.5	48.8	48.2	45.2

In terms of physiologically active matters, WG of oak and beech contain significantly more carotene and chlorophyll than that of conifers. The highest

concentration of vitamin C is found in WG of black pine during spring – 10.9 g/kg<sub>FW</sub> and the lowest in WG of spruce during winter - 2.0 g/kg<sub>FW</sub>. In both conifers, concentration of vitamins are lower in winter than in spring. These seasonal differences are particularly pronounced in the case of vitamin E (Tab. 5).

Table 5: Physiologically active matters in beech, oak, black pine and spruce wood greenery originating from B&H (values are based on fresh weight – F.W.).

Physiologically active matters	Beech		Oak		Black pine		Spruce	
	Spring	Summer	Spring	Summer	Spring	Winter	Spring	Winter
Carotene (mg/kg <sub>FW</sub> )	101.2	52.5	112.3	96	42.9	63.3	54.6	86.1
Vitamin B <sub>2</sub> (mg/kg <sub>FW</sub> )	13	11.2	13.5	11.1	8.8	5.1	9.9	6.5
Vitamin C (g/kg <sub>FW</sub> )	4.9	3.7	4.5	3.1	10.9	5.1	4.2	2.0
Vitamin E (mg/kg <sub>FW</sub> )	128.1	127.5	155.5	162.9	161.4	14.1	111	44.4
Vitamin K (mg/kg <sub>FW</sub> )	21.3	19.9	27.2	24.8	20.3	8.4	28.3	10.8
Chlorophyll (g/kg <sub>FW</sub> )	17.3	13.4	18.3	16.4	12.7	9.1	12.5	9.9

When it comes to the mineral composition of WG, the observed species contain more phosphorus in summer than in other seasons. The same is the case with calcium content in deciduous trees, while the situation is reversed in conifers. Deciduous species are significantly richer in Fe, Mn, Zn and Cu compared to conifers. Wood greenery of oak has by far the most cobalt, while the concentration of molybdenum is more or less balanced (Tab. 6).

Table 6: Macro and micro elements in beech, oak, black pine and spruce wood greenery originating from B&H (values are based on fresh weight – F.W.).

Elements	Beech		Oak		Black pine		Spruce	
	Spring	Summer	Spring	Summer	Spring	Winter	Spring	Winter
Ca (g/kg <sub>FW</sub> )	6.9	9.1	7.7	9.4	12.8	5.5	5.3	4.6
P (g/kg <sub>FW</sub> )	1.2	1.9	0.9	1.6	1.4	1.8	0.9	1.3
Fe (mg/kg <sub>FW</sub> )	951.4	606.6	798.5	488.5	566.6	53.4	159.5	78.1
Mn (mg/kg <sub>FW</sub> )	1818.5	1286.4	995.5	753.2	52.8	33.3	252.9	233.5
Zn (mg/kg <sub>FW</sub> )	181.6	142.2	231	134	31.4	62.5	90.9	44.5
Co (mg/kg <sub>FW</sub> )	17.1	9.6	39.0	23.2	18.4	17.1	9	10.1
Cu (mg/kg <sub>FW</sub> )	10.1	6.9	10.1	5.1	5.8	1.3	4.1	1.2
Mo (mg/kg <sub>FW</sub> )	0.5	0.3	0.7	0.5	0.3	0.2	0.3	0.2

The highest concentration of amino acids was recorded in WG of oak, a total of 47.32 g/kg<sub>FW</sub>, followed by WG of beech – 41.64 g/kg<sub>FW</sub>. Significantly less amino acids were detected in WG of spruce - 33.72 g/kg<sub>FW</sub> and the least in WG of black pine - 31 g/kg<sub>FW</sub> (Tab. 7). Similar relationships are described by Terzić *et al.* (1979) on material from site of

Maoča, in Krivaja river valley in central B&H, with the note that they analyzed samples from two other seasons, summer and autumn. In our case, individually, the most represented amino acid is glutamic acid (in WG of oak), followed by valine (in WG of beech). The least represented amino acid in all samples is methionine (Tab. 7).

Table 7: Aminoacids in beech, oak, black pine and spruce wood greenery, during spring season, originating from B&H (values are based on fresh weight – F.W.).

Aminoacids	Beech	Oak	Black pine	Spruce
	g/kg <sub>FW</sub>			
Alanine	2.47	2.92	1.62	2.13
Arginine	2.32	4.57	1.58	1.93
Aspartic acid	4.41	5.60	2.86	3.29
Glutamic acid	5.00	5.89	3.03	3.95
Glycine	2.28	1.95	1.58	1.90
Histidine	1.10	1.40	0.62	1.13
Isoleucine	2.22	2.65	1.64	2.69
Leucine	3.67	4.35	2.78	3.38
Lysine	2.12	2.51	1.36	2.82
Methionine	0.06	0.19	0.04	0.06
Phenylalanine	2.54	3.01	1.88	2.11
Proline	1.77	2.10	1.23	1.70
Serine	2.18	2.58	1.43	1.77
Threonine	2.13	2.27	1.54	1.63
Tyrosine	1.54	1.64	1.00	1.13
Valine	5.83	3.69	1.99	2.11
Σ	41.64	47.32	26.12	33.73

Woody greenery of black pine is richer in essential oil than WG of spruce. Average yield of pine oil during winter was 0.45% and during spring 0.39%. At the same time, average yields of spruce oil were 0.17% and 0.11%, respectively.

#### 4 CONCLUSIONS

On average, the heaviest WG is discarded by spruce, followed by oak, then black pine and finally beech. At the same time, the greatest absolute and average length has WG of beech, followed by oak, spruce and then black pine. Most leaves/needles in relation to the weight of the basic twig has spruce, then black pine, oak and beech.

In all observed species except spruce, with increasing of twig thickness, the relative share of the *green component* (leaf /needle) decreases. In spruce, with increasing thickness of the basic twig, the relative share of the *green component* slightly increases at

the expense of the other two components (bark and wood). In deciduous trees, this decline is the largest between the first and second sections, while in black pine the largest decline is between the third and fourth sections. In all observed species, the smallest reduction in the share of the *green component* is between the fourth and fifth sections. This means that reducing the thickness of the basic twig from 10 mm to 8 mm, does not contribute to a significant increase in the quality of WG. On the other hand, the inclusion of 8 -10 mm section in WG increases the degree of biomass utilization and reduces production costs.

In deciduous trees the content of chemical elements and compounds takes higher values in spring compared to summer, noting that with vitamins these differences are not large, while with carotene they are emphasized. In conifers, this regularity is less pronounced, when winter and spring are taken as reference seasons. Most proteins contain WG of oak during summer and the least WG of black pine during spring. Conifers contain more than twice as much fat as deciduous trees. The least amount of moisture has WG of spruce during winter, and the highest WG of black pine during spring. WG of oak and beech contain significantly more carotene and chlorophyll than that of conifers. The highest concentration of vitamin C is found in WG of black pine during spring and the lowest in WG of spruce during winter. In both conifers, concentration of vitamins is lower in winter than in spring. These seasonal differences are particularly pronounced in case of vitamin E. Observed species contain more phosphorus in summer than in other parts of the season. The same is the case with calcium content in deciduous trees, while the situation is reversed in conifers. Deciduous species are significantly richer in Fe, Mn, Zn and Cu compared to conifers. Wood greenery of oak has by far the most cobalt, while the concentration of molybdenum is more or less balanced. The highest concentration of amino acids was recorded in WG of oak, followed by beech. Significantly less amino acids were detected in WG of spruce, and the least in WG of black pine. Individually, the most represented amino acid is glutamic acid (in WG of oak), followed by valine (in WG of beech). The least represented amino acid in all samples is methionine. Woody greenery of black pine is richer in essential oil than WG of spruce, either in spring or winter.

From the aspect of production continuity, coniferous wood greenery is of greater importance because it is available throughout the year, and deciduous wood greenery only during the vegetation period. In practice, these differences can be mitigated by choosing the right schedule of felling operations, by cutting down forest stands with deciduous tree species during spring and summer and those with conifers during autumn and winter.

The obtained results can serve as a part of background material in case of new facilities establishment for processing of wood greenery on industrial scale. In this way, an

entirely new product (or more of them) is offered for agriculture, while forestry is finding placement of raw material that has not been exploited until now.

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# CAPÍTULO 4

## THE CHOICE OF OPTIMAL TECHNOLOGY FOR EXTRACTING WOOD GREENERY FROM FOREST DENDROMASS

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**ABSTRACT:** Forests and forest land in Bosnia and Herzegovina encompass an area of 3,231,500 ha out of which 1.28 mill. ha is in the Republika Srpska (RS) entity and the rest in the B&H Federation entity. In the forests of RS each year are cut down around 2.94

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mill. m<sup>3</sup> of dendromass. Of these, about 15-20% or 127,500 to 170,000 m<sup>3</sup> makes wood greenery - twigs with needles or leaves, with 10 mm diameter at thick end, measured with bark. Based on the experience of others, first of all from Russia, the Baltic and Scandinavian countries, it is known that wood greenery represents valued raw material in different sectors of economy: agriculture, pharmaceutical and cosmetics industry and others. The entire annual attack of wood greenery remains unused, with us. There are at least two reasons for this: a) because there are still no elaborated and proven practices of rational collection and concentration of raw materials to processing plants; and b) because we are not familiar enough with the methods of wood greenery processing. A detailed analysis of the technology of wood assortment production in our forestry practice has been carried out. Based on these findings, a model of integrated forest utilization was conceived, including wood greenery. Then, the technology of wood greenery processing was analyzed in the following sense: mechanical drying and fractionation processes, chemical extraction processes, thermal processes by classical heating and energy use of the microwave electromagnetic field and their combinations: mechanical-chemical methods and thermo-chemical methods. Finally, such technology of wood greenery processing has been selected to optimally matches with the integrated use of forest resources, in terms of the following

criteria: minimal negative environmental impact, maximum safety at work, low energy consumption, primarily from renewable sources, maximum quality of final products.

**KEYWORDS:** Wood greenery. Processing technology. Forest dendromass.

## LA ELECCIÓN DE LA TECNOLOGÍA ÓPTIMA PARA LA EXTRACCIÓN DE MADERA VERDE DE DENDROMASA FORESTAL

**RESUMEN:** Los bosques y las tierras forestales de Bosnia y Herzegovina abarcan una superficie de 3.231.500 ha, de las cuales 1,28 millones de ha se encuentran en la entidad de la República Srpska (RS) y el resto en la entidad de la Federación de ByH. En los bosques de la RS se talan cada año unos 2,94 millones de m<sup>3</sup> de dendromasa. De ellos, alrededor del 15-20%, es decir, entre 127.500 y 170.000 m<sup>3</sup>, son de madera verde, es decir, ramitas con agujas u hojas, con un diámetro de 10 mm en el extremo grueso, medido con corteza. Basándose en la experiencia de otros, en primer lugar, de Rusia, los países bálticos y escandinavos, se sabe que la madera verde representa una materia prima valiosa en diferentes sectores de la economía: agricultura, industria farmacéutica y cosmética y otros. La totalidad del ataque anual de madera verde permanece sin utilizar, con nosotros. Esto se debe al menos a dos razones: a) porque todavía no existen prácticas elaboradas y probadas de recogida racional y concentración de materias primas para las plantas de procesamiento; y b) porque no estamos suficientemente familiarizados con los métodos de procesamiento de la madera verde. Se ha llevado a cabo un análisis detallado de la tecnología de producción de surtido de madera en nuestra práctica forestal. Sobre la base de estos resultados, se concibió un modelo de aprovechamiento forestal integrado que incluye la madera verde. A continuación, se analizó la tecnología de procesamiento de madera verde en el siguiente sentido: procesos de secado y fraccionamiento mecánicos, procesos de extracción química, procesos térmicos por calentamiento clásico y uso de energía del campo electromagnético de microondas y sus combinaciones: métodos mecánico-químicos y métodos termoquímicos. Finalmente, esta tecnología de procesamiento de madera verde ha sido seleccionada para que coincida de manera óptima con el uso integrado de los recursos forestales, en términos de los siguientes criterios: mínimo impacto ambiental negativo, máxima seguridad en el trabajo, bajo consumo de energía, principalmente de fuentes renovables, máxima calidad de los productos finales.

**PALABRAS CLAVE:** Madera verde. Tecnología de procesamiento. Dendromasa forestal.

### 1 INTRODUCCIÓN

Wood greenery (WG) consists of branches with needles or leaves with a diameter of up to 10 mm measured over bark from the butt end (Figure 1); it is also referred as technical greenery. WG is made from brushwood of coniferous and hardwood trees, whereby brushwood we mean dendromass less than 7 cm thick, measured over bark, while branchwood is thicker than 7 cm, measured over bark.

WG is a raw material for the production of several products with a variety of usable values such as: vitamin-mineral flour, chlorophyll-carotenoic paste, essential

oils, fitoinsecticides, plant growth regulators, phytopharmaceuticals, primary forms of medicinal preparations in human medicine etc.

Figure 1: Wood greenery of fir (*Abies alba* Mill.); (photo: S. Ljubojević)



Forests and forest land in Bosnia and Herzegovina encompass an area of 3,231,500 ha out of which 1.28 million ha is in the entity of Republika Srpska (RS) and the rest in the entity of Federation B&H. In the forests of RS about 2.94 mil. m<sup>3</sup> of dendromass are cut down every year. Of these, about 15-20% or 127,500 to 170,000 m<sup>3</sup> makes WG (Ljubojević *et al.*, 2007). Apart from forest records, the determination of the amount of logging residues can also be done using dendrometric formulas (Daniš *et Neruda*, 2021). For now, the entire annual attack of WG remains unused. There are at least two reasons for this: a) because there are still no elaborated and proven practices of rational collection and concentration of raw materials to processing plants; and b) because we are not familiar enough with the methods of WG processing.

The aim of the paper is to analyze the technology of production of wood assortments in our forestry practice and based on this knowledge, to propose the concept of integral forest exploitation, which includes WG. Then to analyze the technologies of WG processing and to choose the solution that most completely satisfies the given criteria.

## 2 MATERIAL AND METHODS

The subject of research is WG of domestic conifers: fir (*Abies alba* Mill.), spruce (*Picea excelsa* Link.), Scots pine (*Pinus silvestris* L.) and black pine (*Pinus nigra* Arnold). In comparison to the raw material of the broadleaf (deciduous) species, the coniferous raw materials are of no seasonality and can be used throughout the year.

Table 1: The basic characteristics of the observed objects.

Basic characteristics	Observed objects			
Location	Industrial plantations „INCEL“	Forest estate „Borja“ Teslić	Forest estate „Gostović“ Zavidovići	Forest economy region Kneževo
Type of ownership	Joint-stock company	State-owned enterprise		Private property
Forest type	Pine plantations	High mixed beech, fir and spruce forest	High forest and plantations of pines	High forest of spruce
Means of work	Working phase - harvesting			
	Harvester FMG Lokomo 990	Chainsaw		
	Working phase - wood bunching and extracting			
	Skidder LKT- 81	Skidder LKT-80	Ackja and Gantner HSW 20 winches joined in a cableway system	Winch TPS TUN 40 mounted on tractor Ursus C360
Harvesting system	Assortment method	Assortment method	Full-tree method	Full-length method
Appearance form of wood greenery (WG)	WG integrated with branchwood - at felling site	WG integrated with branchwood - at felling site	WG integrated with branchwood - at timber yard	WG integrated with branchwood - at felling site

An analysis of the production technology of wood assortments in our forestry practice was carried out by monitoring four felling areas in different harvesting season. According to the time of realization, there are winter felling (from October 1 to March 31) and the summer felling (from April 1 to September 30). The basic characteristics of the observed objects are given in Table 1.

Harvesting and processing technology was observed using method of continuous timing during one full working day per felling area. In this way, a chronological sequence of work elements was established, from the moment when the cutting begin until the moment when the timber assortments are dispatched to the timber yard.




Along with this, not disturbing the regular production process, three organizational solutions were tested.

1. WG was separated from the branchwood and brushwood at felling sites using simple garden tree shearing scissors, then put in 70-liter bags, loaded on pack-horses and carried to the storage place along the truck road.
2. Brushwood was separated from the branchwood at felling sites using axes and strong bypass lopper and then stacked in small bundles. From here the raw material was handed over to the iron baskets arranged along the skid road. In regular production these baskets are used for the transportation of stacked wood. Baskets were mounted on the front and rear of the skidder and forwarded to the storage place, where the final separation of WG from brushwood was carried out.
3. The third variant involves specific felling operation, which is rarely encountered in our forestry practice. It is a strip clear cut in Scots pine plantation on a steep terrain, where it is not justified to build permanent roads (skidding trucks and forest roads). Trees were cut and hauled as a whole or split by half, using pair of winches (Figures 2-5). WG was separated from the branchwood and brushwood by hand tools at the end of the ropeway.

To better understand the working conditions on preparation of WG, the average moisture content by the tree species and the felling season was determined, in the usual way and based on small samples ( $n_j \leq 30$ ). For the same reasons, the bulk density of WG was evaluated as well. With that regard, a wooden container of 1 x 0.5 x 0.5 m was made. It was filled with material to the top and weighed on the field scale, with a reading of 0.1 kg. The obtained values are converted to the standard unit  $\text{kg}/\text{m}^3$ .

By inspecting the scientific and professional papers, technical documentation and patents, processing technologies of WG have been analyzed and a solution that satisfies the following criteria has been selected: a) a mass-usable product made without further fractionation, b) waste that is generated during production is usable, c) production takes place without use of chemical agents, d) production is flexible with respect to raw material origin - it is possible in mobile and stationary plants, e) selected solution is proven in practice. Also, chosen solution does not adversely affect the environment, provides all the necessary safety at work and requires low energy consumption, primarily from renewable energy sources.

Figures 2-5: Whole trees haulage using pair of winches - Ackja winch for skidding from stumps to theropeway and Gantner HSW 20 winch for extraction down the mainline (photo: S. Ljubojević).

Fig. 2: Sledge winch Ackja	Fig. 4: A view at the clear cut strip in Scots pine plantation	Fig. 5: Load attached to the carriage with clamping device
 <p data-bbox="138 755 397 807">Fig. 3: Sledge winch Gantner HSW 20</p>		

### 3 RESULTS

To make WG a kind of raw material, it is necessary to conduct several working operations: to collect branchwood from the felling site, to separate WG from the branchwood and to gather it in a form suitable for transportation, to transport it to the processing site. In doing so, we distinguish between internal transport, on a move from stumps to the storage place, and external transport, from the storage place to the processing plant.

Collecting WG from standing trees is not reasonable for reasons that need not to be specifically explained. On the contrary, the only possible solution is the separation of the tree's greenery immediately after the tree felling in any situations: regular felling, sanitation felling, random yield etc.

Possible negative ecological impacts induced by the crown mass removal, can be reduced by appropriate timing of operations, minimizing the nutrient removals from the forest sites and recycling of ash from the combustion installation (Ghaffariyan, 2010; Kuiper, 2006).

### 3.1 MANUAL PREPARATION OF WOOD GREENERY

In a typical case, manual preparation of WG involves the following work elements:

- collecting branchwood left behind after cutting the trees,
- separation of WG from branchwood,
- carrying of WG to the containers for transport,
- stacking of WG at the containers for transport.

The following average working time structure was determined for the implementation of the described work elements in the eighth hours working day (WD):

- Effective working time (EWT) = 6 h (75% of WD)
- Delay time (DT) = 2 h (25% of WD),

where under the EWT we mean a period of useful work, and under the DT allowed breaks in work, such as: mealtime, rest and personal time, interference time.

The following average structure of EWT was determined:

- Preparatory time = 14.4 min (4% of WT)
- Time of transition (from tree to tree) = 24.8 min (8% of WT)
- Separation of WG from branchwood = 277.2 min (77% of WT)
- Disposal and stacking of WG at the container = 39.6 min (11% of WT)

The following average production rate per worker was determined: 161.4 kg of WG per WD, with variation interval between 134 - 189 kg of WG per WD. These results point to the conclusion that manual preparation of WG is not economically justified due to the low productivity of manual labor.

### 3.2 INTERNAL TRANSPORT

Relatively high moisture content in fresh WG and its low bulk density (Table 2) make it difficult to find rational solutions with the transport means currently in use in our forestry, whether the raw material is carrying from the forests in bags on pack-horses, or in iron baskets mounted on the skidder. The only economically viable solution is to transport entire trees using wire-rope systems (Figures 2-5). However, this technological solution rarely meets our forestry practice, and as a such, it does not have a decisive significance in the eventual organization of mass production. Conversely, mechanical hauling of whole trees or parts of them is not acceptable due to the high pollution and damage of raw material (Ljubojević, 2008).

Table 2: Moisture content in fresh wood greenery and its bulk density.

Harvesting season	Tree species			
	Scots pine	Black pine	Fir	Spruce
Moisture content (%)				
Winter	51	51	50	48
Summer	51	53	50	47
Bulk density (kg/m <sup>3</sup> )				
Winter/summer	57,6	82	142	173,2

### 3.3 CONCEPT OF PRIMARY COLLECTION AND CONCENTRATIONS OF WOOD GREENERY TO THE PROCESSING SITE

An applicable techno-economic solution assumes the mechanized gathering and palletizing of forest residues in the form of biomass bundles and their delivery to the processing plants (Illustrated table 1, production lines 1 & 2). In this way, it significantly shortens the time of primary collection and concentration of the raw material to the processing site, ensures better raw material quality and at the same time increases the bulk density of the load. Two-stage separation of biomass is performed at the processing site. In the first step, branchwood and brushwood are separated by a light hydraulic crane. Branchwood is directed to the production of solid biofuels (woodchips, briquettes, pellets). Brushwood is mechanically separated into ligno-cellulose skeleton and wood greenery (Figure 6).

Illustrated table 1: Production lines for bundling of logging residues in regular felling.

1	Logging residues at the felling site	Making bundles with the slash bundler at the felling site	Collecting bundles over felling site and transferring to the storage place	Transporting bundles to the processing plant
				

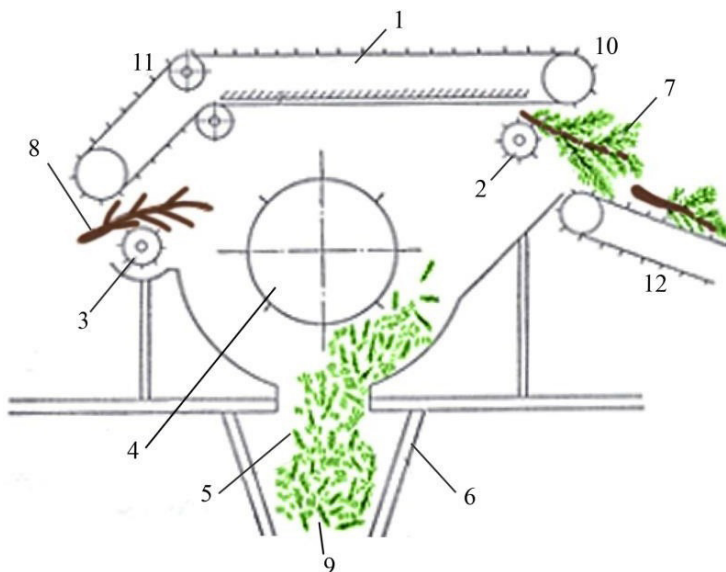


2	Logging residues at the felling site	Collecting and transporting forest residues to the storage place	Making bundles with the slash bundler at the storage place	Transporting bundles to the processing plant
				

(source: Ljubojević, 2016).

Separation of WG from the wooden skeleton is the most important operation in the phase of WG preparation. By switching from manual to mechanized production, it significantly increases productivity and reduces production costs (Hakkila, 1989; Levin *et al.*, 1984; Nikitov, 1985). Separator “ODZ-12A” (ODZ is an acronym of “Отделитель древесной зелени” [in Russian]) is an automatic machine constructed and introduced into regular production in the former USSR. The Separator consists of five basic parts and mechanisms mounted on a rigid chassis: 1. Belt conveyor (internal), 2. Roller inserter, 3. Roller ejector, 4. Separation drum with blades, 5. Bunker for receiving WG, 6. Chassis (Figure 6).

Figure 6: Separator of wood greenery “ODZ – 12A”: 7. Brushwood, 8. Bare branches, 9. WG, 10. Powered pulleys, 11. Tensile rowels, 12. Belt conveyor (external) for the delivery of WG in front of the separator (positions 1- 6 are described in the text); (according to Tomčuk *et Tomčuk*, 1966).



Automatic feeding of separation drum with WG and discharge of bare branches is carried out by Belt conveyor /1/. Top layer of belt consists of metal rolls with welded ribs- a thin cylinder in the form of non-head nails. Its working position and strength are regulated by Tensile rowels /11/. Drum with blades /4/ is the main part of Separator. It divides green parts from wooden skeleton by means of knives fixed with clamps. Rubber shock absorbers prevent collisions during rotation. External belt conveyor /12/ inserted WG in a shape of brushwood into the Separator, piece per piece with a thicker end forward.

Main technical characteristics of the Separator are: capacity - 1.5 t WG/h, separation purity - 98%, power - 3.8 kW, drum speed 500 – 650 rotations /min, working volume of bunker - 0.5 m<sup>3</sup>.

### 3.4 CHOICE OF OPTIMAL WOOD GREENERY PROCESSING TECHNOLOGY

WG serves as a raw material for obtaining a large number of products with a wide usage value, for which various technological processes have been developed (Table 3). Otherwise, fresh (unprocessed) branches with needles are used as a protective cover for young agricultural crops, soil protection from moisture loss, as an addition to fodder and as a raw material for compost production (Duryea *et* Edwards, 1997; Daugavietis *et al.*, 2015). When domestic animals and poultry are fed, a previous smoothing with water vapor is practiced.

When the simplified review of processing technologies is filtered through the set of criteria, the production of vitamin-mineral flour (VMF) comes out as an optimal solution. VMF can be produced in mobile facilities and in stationary plants, both of which operate according to the same principles (Hakkila, 1989; Levin *et al.*, 1984; Nikitov, 1985; Tomčuk *et* Tomčuk, 1966). Figure 7 shows the technological scheme of VMF production. The exception is only WG of fir, which due to the high content of essential oil requires additional technological intervention, combining hydrodistillation with short-term processing of the raw material by high pressure steam (so-called steam cracking) (Levdanskij *et al.*, 1992).

Table 3: Simplified review of wood greenery processing technologies.

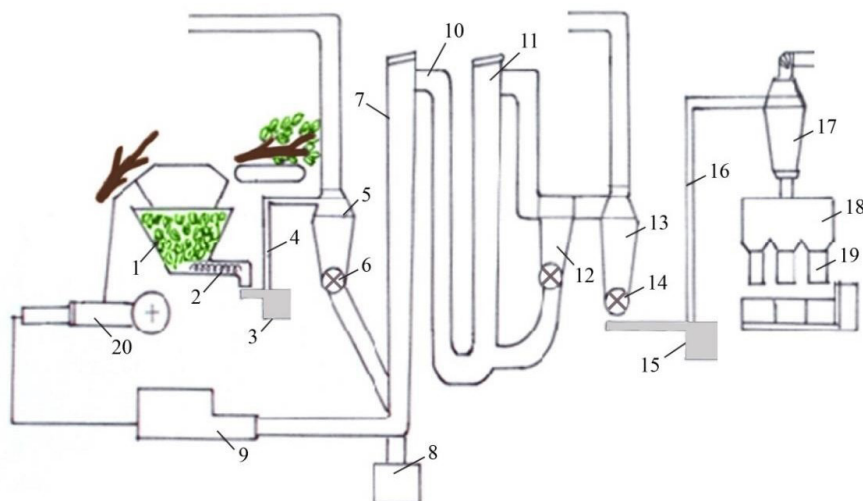
Products obtained from wood greenery	Essence of the processing technology	Source
Vitamin-mineral flour	Thermal and mechanical treatment by combining drying, sorting and grinding processes	Hakkila (1989), Levin (1984) Nikitov (1985), Tomčuk (1966)
Essential oils	Hydrothermal treatment by aqueous distillation	Daugavietis (2014), Tomčuk (1966)

Biologically active compounds (BAC) of low molecular weight	Chemical treatment by extraction with aqueous sodium hydroxide solution <sup>*/</sup>	Karmanova (2005), Terenteva (2016), Šanina (2004)
BAC of high molecular weight	Thermochemical treatments	Karmanova (2005), Terenteva (2016)
Chlorophyll-carotenoic paste	Thermochemical treatment by gas extraction process in combination with distillation of essential oils	Levin (1984), Tomčuk (1966)
Primary forms of medicinal preparations in human medicine	An infusion in water or in diluted ethanol; coniferous therapeutic extract	Levin (1984), Paršikova (2015)

<sup>\*/</sup> This process yields more useful products. Lipids are used in perfumes, medicine and other industries. Concentrate of neutral ingredients (provitamin concentrate), separated by emulsion extraction, can be used as a bioactive additive in the perfumery and cosmetic industry and as an additive in domestic poultry and cage breeding fur animals.

Production of VMB is carried out according to the following procedure (Figure 7): WG from the Collecting bunker /1/ drops onto the Screw conveyor /2/ which brings it to the Crusher /3/. Rough chips come out of the Crusher as a mixture of needles and particles of wood and bark. It is carried through Conveying duct /4/ by compressed air into the Bunker /5/. At the bottom of the Bunker there is a Dosing unit /6/ by which rough chips is evenly distributed to the First drying column /7/. A mixture of greenery and particles of wood and bark floats in the column space. Stream of hot air is programmed so that only the hardest fraction (particles of wood) can fall down. This fraction is collected into the Container below the First drying column /8/, from where it is periodically inserted into the Furnace /9/. From the First column, the dried material goes to the Second /10 / and the Third column /11/ for further drying. A part of the material that is not sufficiently dried and, therefore is heavier, is separated /12/ and via feedback sent for redrying. Completely dried material goes through the Cyclone /13/ and the Dosing unit /14/ to the Mill /15/ in which it turns into flour. It passes through a Sieve of 1.5-2 mm diameter and through Conveying duct /16/ goes to the Cyclone /17/ with WMF Bunker /18/ and with Line for filling and sewing bags with VMF /19/. Bare branches fall into the Press for briquettes /20/ which are intended for market or are used on-site for own needs.

Figure 7: Technological scheme for the production of vitamin-mineral flour: 1. Bunker for receiving WG, 2. Screw conveyor, 3. Crusher, 4. Pneumatic conveyor, 5. Bunker for receiving rough chips, 6. Dosing unit, 7. First drying column, 8. Bunker for receiving waste material from the first drying column (wood and bark particles), 9. Furnance, 10. Second drying column, 11. Third drying column, 12. Separator of residual wet material, 13. Cyclone, 14. Dosing unit, 15. Mill, 16. Pneumatic conveyor, 17. Cyclone, 18. Bunker of VMF, 19. Line for filling and sewing bags with VMF (according to Tomčuk et Tomčuk, 1966).



Compared with the above-mentioned plant, Hakkila (1989) describes a slightly smaller, more compact and more mobile chip sorter SIKO-2. Recently, processing of WG into VMF using the energy of the microwave electromagnetic field, have been described by Posmetjev et Latiševa (2018). The essence of this process is to convert the energy of the microwave electromagnetic field into the heat. According to the authors, with applying their solution one can achieve separation purity of 90-95 % by weight without prior mechanical processing (as made by „ODZ 12A“ separator). Unfortunately, this solution has not yet lived in practice, inspite the fact that it has been officially presented in April 2006.

## 4 CONCLUSIONS

To become a raw material, it is necessary to conduct several operations over WG: to gather branchwood over felling site, to separate WG from branchwood, to gather it in a form suitable for transport and to deliver it to the processing site.

Manual preparation of WG is not economically justified due to the low productivity of manual labor - 161.4 kg of WG per worker and WD, with variation interval between 134 - 189 kg of WG.

Relatively high moisture content in fresh WG and its low bulk density make it difficult to find rational solutions with the transport means currently in use in our forestry,

whether the raw material is carrying from the forests in bags on pack-horses, or in the iron baskets mounted on the skidder. The only economically viable solution is to transport entire trees using wire-rope system. However, this technological solution rarely meets our forestry practice, and as a such, it does not have a decisive significance in the eventual organization of mass production.

An applicable techno-economic solution assumes the mechanized gathering and palletizing of forest residues in the form of biomass bundles and their delivery to the processing plants. In this way, it significantly shortens the time of primary collection and concentration of the raw material to the processing site, ensures better raw material quality and at the same time increases the bulk density of the load.

Separation of greenery from the wooden skeleton is the most important operation in the phase of preparation. By switching from manual to mechanized production, it significantly increases productivity and reduces production costs.

Of the many products that can be obtained from WG, VMF is especially distinguished as a mass-consumable product in the feeding of domestic animals. Its production is flexible in relation to the source of raw materials - it is possible in mobile and stationary plants, and waste generated during production is usable. Production takes place without the use of chemicals and without negatively affecting the environment.

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# CAPÍTULO 5

## PLAN DE ACCIÓN PARA LA PROMOCIÓN DEL BAGRE ARMADO (HYPOSTOMUS PLECOSTOMUS) EN VILLAHERMOSA TABASCO

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**RESUMEN:** El propósito de este proyecto es desarrollar un plan de acción para la promoción del bagre armado (*Hypostomus plecostomus*) en Villahermosa, Tabasco. El bagre armado es un modelo de estudio importante para la investigación en diversas áreas como la biología, la ecología y la genética. Su rápido crecimiento y su capacidad para adaptarse a diferentes ambientes lo convierten en un organismo ideal para la investigación científica. Este proyecto se enfoca en la identificación de las oportunidades de mercado y la creación de estrategias efectivas para fomentar el consumo y comercialización del bagre armado. La metodología incluye un análisis de mercado, entrevistas y la implementación de campañas publicitarias dirigidas. Los resultados esperados son un incremento en la demanda local del bagre armado y una mayor participación de los productores locales en el mercado. La relevancia de este proyecto radica en su potencial para mejorar la economía local, apoyar a los pescadores y promover prácticas sostenibles de pesca.

**PALABRAS CLAVE:** Plan de Acción. Promoción. Bagre armado. Modelo de estudio. Prácticas Sostenibles de Pesca.

## ACTION PLAN FOR THE PROMOTION OF ARMED CATFISH (HYPOSTOMUS PLEcostOMUS) IN VILLAHERMOSA TABASCO

**ABSTRACT:** The purpose of this project is to develop an action plan for the promotion of the plecostomus catfish (*Hypostomus plecostomus*) in Villahermosa, Tabasco. The plecostomus catfish is an important study model for research in various areas such as biology, ecology and genetics. Its rapid growth and ability to adapt to different environments make it an ideal organism for scientific research. This project focuses on identifying market opportunities and creating effective strategies to encourage the consumption and marketing of plecostomus catfish. The methodology includes a market analysis, interviews and the implementation of targeted advertising campaigns. The expected results are an increase in the local demand for plecostomus catfish and a greater participation of local producers in the market. The relevance of this project lies in its potential to improve the local economy, support fishermen and promote sustainable fishing practices.

**KEYWORDS:** Action Plan. Promotion. Armed Catfish. Study Model. Sustainable Fishing Practices.

### 1 INTRODUCCIÓN

El bagre armado desempeña un papel crucial en las comunidades pesqueras de Villahermosa, Tabasco, y en toda la región circundante. Este pez de agua dulce no solo es una fuente de alimento esencial para muchas familias locales, sino que también constituye un importante motor socioeconómico en la zona.

El estudio explora el contexto actual del mercado, identifica barreras, oportunidades, y propone estrategias específicas para incrementar la aceptación y el consumo de este pez, promueve prácticas sostenibles de pesca. La metodología utilizada en este estudio incluyó un enfoque mixto. Las encuestas proporcionaron datos numéricos sobre la frecuencia y cantidad de consumo, mientras que las entrevistas ayudaron a comprender las razones de estos patrones de consumo.

La promoción del bagre armado en Villahermosa es crucial debido a su potencial económico y ecológico.

La percepción negativa limita su aprovechamiento y conservación.

Abordar este tema radica en la necesidad de conservar la biodiversidad y potenciar recursos locales subvalorados. como en otras regiones, el bagre armado ha sido objeto de controversia debido a su impacto ambiental y a su reputación como especie invasora.

A pesar de ello, esta especie posee un potencial económico considerable, tanto en términos de pesca deportiva como en el control de algas y limpieza de cuerpos de



agua al promover un enfoque integral y sostenible hacia esta especie, se podrá garantizar su aprovechamiento responsable y su contribución al bienestar humano y ambiental a largo plazo.

El pez bagre armado *Hypostomus plecostomus*, es una especie de pez que se ha introducido en los cuerpos de agua de México, incluyendo la región de Tabasco. A pesar de ser una especie invasora, ha encontrado un lugar en la gastronomía local de Villahermosa debido a su abundancia en los ríos y lagos de la región.

Tradicionalmente, el pleco no era una especie de gran valor culinario en la región. Sin embargo, debido a su abundancia y a la necesidad de gestionar su población, los habitantes locales comenzaron a incorporarlo en sus prácticas culinarias.

La gastronomía de Villahermosa ha incorporado el Bagre Armado en varios platillos tradicionales. Entre los más destacados se encuentran:

- **Caldo de Pleco:** Un caldo nutritivo que se prepara con verduras locales y especias.
- **Pleco Asado:** Preparado a la parrilla, marinado con hierbas y especias de la región.

La inclusión del Bagre Armado en la dieta local también tiene un impacto positivo en la sostenibilidad. Al consumir esta especie invasora, se ayuda a mantener el equilibrio en los ecosistemas acuáticos de Tabasco.

Estas incluyen programas de pesca dirigida, campañas de sensibilización y estudios para evaluar la efectividad de métodos de control biológico.

La inclusión del Bagre en la gastronomía local sigue siendo una tendencia creciente. Chefs locales y restaurantes han comenzado a experimentar con nuevas recetas y técnicas de preparación para hacer más atractiva la carne de pleco. Esto no solo ayuda a controlar su población, sino que también diversifica la oferta culinaria de la región.

La carne del Pez Bagre Armado es baja en grasas saturadas, lo que la convierte en una opción saludable para las personas que buscan reducir su ingesta de grasas no saludables.

## 2 POBLACIÓN O UNIVERSO / MUESTRA

Población: De acuerdo con el Instituto Nacional de Estadística y Geografía (INEGI), la población del municipio de Centro, en el estado de Tabasco, es de 683,607 habitantes.

Muestra: Para calcular el tamaño de la muestra con un nivel de confianza del 95%, margen de error de 6.81 y una población de 683,607 habitantes se realizó la siguiente formula:

$$n_0 = \frac{n_0}{1 + \left(\frac{n_0 - 1}{N}\right)}$$

### 3 TIPO DE ESTUDIO:

Se optó por un enfoque mixto (cualitativo y cuantitativo) para combinar datos numéricos con percepciones subjetivas y proporcionar un análisis de los patrones de consumo y preferencias de compra.

Los datos cuantitativos proporcionaron información numérica sobre la frecuencia de consumo, las preferencias y las tendencias, lo cual fue fundamental para comprender el panorama general del mercado. Por otro lado, los datos cualitativos permitieron explorar en profundidad las percepciones, opiniones y experiencias de los posibles consumidores.

### 4 DESCRIPCIÓN DEL INSTRUMENTO:

Se creó un cuestionario estructurado que incluyó un total de 18 preguntas, combinando opciones de respuesta múltiple con preguntas abiertas, con el fin de abordar el consumo y los diferentes hábitos relacionados al Pez Bagre Armado. Este instrumento fue diseñado para ser administrado de manera eficiente a través de la plataforma Google Forms, facilitando así la recolección de datos de una muestra representativa de la población objetivo.

### 5 PROCEDIMIENTO DE RECOLECCIÓN:

Se llevó a cabo la recolección de datos, utilizando la plataforma Google Forms como herramienta principal para administrar el cuestionario, este se distribuyó ampliamente a través de diversas plataformas digitales, como redes sociales, grupos comunitarios y correos electrónicos, con el objetivo de alcanzar a una muestra diversa de la población. El cuestionario incluye preguntas demográficas, de frecuencia de consumo, preferencias y factores determinantes en la decisión de compra.

### 6 PREGUNTAS DEL INSTRUMENTO:

**Se formularon preguntas sobre la percepción y posibles consumos del pez bagre armado.**

## **Cuestionario sobre hábitos de consumo del Pez Bagre Armado**

Este cuestionario tiene como objetivo el recopilar información detallada sobre el consumo y los diferentes hábitos relacionados al Pez Bagre Armado entre los residentes de Villahermosa, Tabasco.

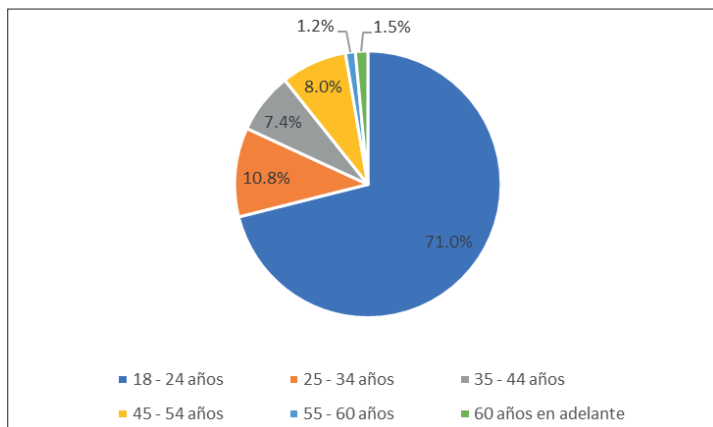
### **7 ANÁLISIS DE DATOS:**

#### **7.1 ANÁLISIS DESCRIPTIVO:**

Los datos recolectados fueron resumidos y analizados utilizando Microsoft Excel, empleando herramientas de análisis estadístico para identificar patrones y tendencias sobre el conocimiento y consumo del pez bagre armado. Este proceso incluyó la limpieza de datos, el cálculo de estadísticas descriptivas, la creación de visualizaciones y el análisis comparativo de subgrupos.

Se realizó un análisis segmentado de los datos por variables demográficas clave para entender mejor los conocimientos y patrones de consumo del pez bagre armado.

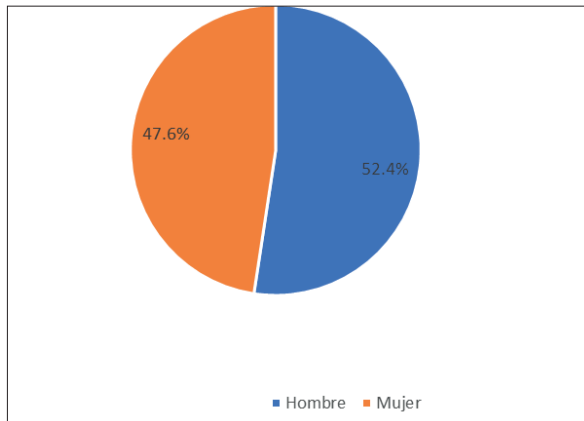
#### **1. ¿Cuál es su rango de edad?**



La gráfica muestra la distribución de edades de una población encuestada, con una clara predominancia de jóvenes adultos. El 71.0% de los encuestados tiene entre 18 y 24 años, mientras que el 10.8% está en el rango de 25 a 34 años. Los participantes de 35 a 44 años constituyen el 7.4%, y aquellos de 45 a 54 años representan el 8.0%. Los grupos de edad de 55 a 60 años y de 60 años en adelante son significativamente menores, con un 1.2% y un 1.5%, respectivamente.

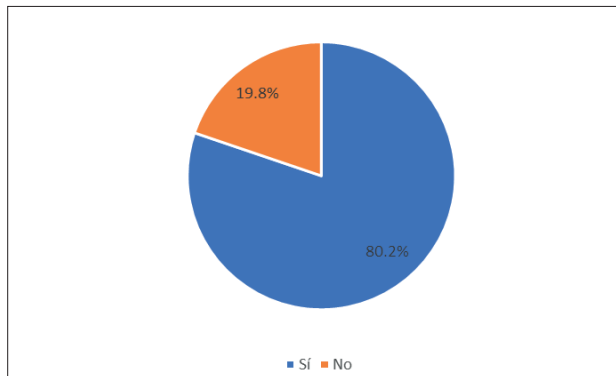
Esta distribución sugiere que la muestra está fuertemente inclinada hacia los jóvenes, con una representación mucho menor de personas de mayor edad, lo que podría influir en los resultados y conclusiones del estudio si la edad es un factor relevante.

## 2. ¿Cuál es su género?



La gráfica presenta la distribución de género de una población encuestada, donde el 52.4% son hombres y el 47.6% son mujeres. Esta distribución indica una ligera predominancia masculina, con una diferencia de 4.8 puntos porcentuales en comparación con las mujeres. A pesar de esta diferencia, la muestra está relativamente equilibrada en términos de género, lo que sugiere una representatividad razonable de ambos géneros en el estudio.

## 3. ¿Conoces o has escuchado hablar acerca del pez bagre armado mejor conocido como (Pez Diablo)?



La mayoría de las personas encuestadas, un 80.2%, están familiarizadas con el pez bagre armado, también conocido como Pez Diablo. Sin embargo, un 19.8% no tiene conocimiento sobre este pez.

Para evaluar la percepción del bagre armado en medios y redes sociales, primero se implementaron herramientas de monitoreo de medios y redes sociales como

Instagram, Twitter, Facebook y Google, con el fin de rastrear menciones del bagre armado en diversas plataformas de medios y redes sociales.

Estas herramientas permitieron captar en tiempo real las conversaciones y publicaciones relacionadas con el bagre armado, proporcionando una amplia base de datos sobre las percepciones públicas y los temas discutidos.

Para identificar desafíos y oportunidades para el bagre armado se revisaron los datos segmentados.

Se verificaron y consolidaron los datos provenientes de artículos científicos, informes técnicos, publicaciones gubernamentales, encuestas, entrevistas y observaciones participantes, lo que proporcionó una base de datos robusta y comprehensiva.

Para elaborar estrategias para la promoción del bagre armado se abordaron los principales desafíos identificados, como la sobreexplotación y la percepción pública negativa, y para aprovechar las oportunidades detectadas, como la promoción de prácticas sostenibles y la educación comunitaria.

## 8 ESTRATEGIAS:

### ➤ **Educación y Sensibilización Comunitaria**

1. Organizar talleres y seminarios en comunidades locales para educar a pescadores, comerciantes y consumidores.
2. Desarrollar y distribuir materiales educativos (folletos, infografías, videos) que destaquen la importancia de la conservación del bagre armado y las mejores prácticas de pesca sostenible.

### ➤ **Mejora de la Comercialización y Valor Agregado**

1. Desarrollar campañas de marketing digital y eventos que resalten las cualidades del bagre armado, tales como su sabor y sus beneficios nutricionales, para atraer a nuevos consumidores.
2. Fomentar la creación de productos derivados del bagre armado (como filetes, Tacos, Alimento para mascotas entre otros) que puedan venderse a precios más altos y en mercados más amplios.

### ➤ **Promoción de Prácticas de Pesca Sostenible**

1. Capacitar a los pescadores en técnicas de pesca sostenible, como el uso de redes selectivas que reduzcan la captura incidental y la implementación de temporadas de veda.

2. Colaborar con autoridades locales y organizaciones de conservación para monitorear y regular las prácticas de pesca, asegurando el cumplimiento de las normativas.

➤ **Fortalecimiento de las Redes de Colaboración**

1. Establecer y fortalecer alianzas entre pescadores, investigadores, organizaciones no gubernamentales y autoridades locales para la gestión sostenible del bagre armado.
2. Fomentar la investigación continua sobre el bagre armado y su ecosistema, promoviendo proyectos colaborativos que involucren a universidades e instituciones de investigación.

➤ **Fomento del Turismo y Ecoturismo**

1. Desarrollar rutas turísticas y experiencias educativas que incluyan actividades como la observación de la pesca sostenible del bagre armado.
2. Promover el ecoturismo como una alternativa económica que beneficia tanto a las comunidades locales como a la conservación del bagre armado.

➤ **Investigación Continua**

1. Encuestas y estudios de seguimiento Realizando encuestas periódicas para monitorear cambios en los patrones de consumo y efectividad de las estrategias implementadas.

## 9 RESULTADOS

Se definió el alcance de la investigación, considerando aspectos como la morfología, ciclo de vida, comportamiento reproductivo, alimentación y hábitat del bagre armado. También se incluyeron sus interacciones ecológicas, su papel en el ecosistema, y su distribución geográfica. Adicionalmente, se evaluaron los impactos ambientales, tanto positivos como negativos, su valor económico en la pesca comercial y el consumo humano, y las percepciones sociales relacionadas con esta especie.

Se realizó una investigación de campo para recolectar datos primarios a través de encuestas y entrevistas dirigidas a pescadores, comerciantes, consumidores, expertos y observación participante en comunidades, con el fin de obtener una comprensión directa de la percepción actual y las prácticas relacionadas con el bagre armado.

De igual manera se realizó un informe Técnico: Plan de Acción para la Promoción del Bagre Armado en Villahermosa, Tabasco.

## 10 CONCLUSIONES

La transformación de la percepción del bagre armado en Villahermosa, Tabasco, requiere un enfoque integral que combine la educación, la promoción gastronómica y las alianzas estratégicas. Las estrategias propuestas en este informe están diseñadas para abordar los desafíos identificados y capitalizar las oportunidades, promoviendo así un manejo sostenible y una mayor aceptación del bagre armado.

## 11 RECOMENDACIONES

Es esencial desarrollar y promover campañas educativas que proporcionen información precisa sobre la biología, comportamiento y beneficios del bagre armado. Estas campañas deben dirigirse a diferentes segmentos de la población, utilizando medios digitales y tradicionales para asegurar un amplio alcance.

Implementar estrategias para promover prácticas de consumo sostenibles del bagre armado. Esto incluye la creación de programas de capacitación para pescadores y consumidores sobre métodos sostenibles de captura y consumo.

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# CAPÍTULO 6

## FITOHORMONAS Y FERTILIZACIÓN QUÍMICA EN LA RENTABILIDAD DE LA PRODUCCIÓN DE SEMILLA DE PASTO BUFFEL ZARAGOZA 115 EN DOS ESTACIONES DEL AÑO BAJO RIEGO EN EL NORTE DE COAHUILA, MEXICO<sup>1</sup>

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**RESUMEN:** El objetivo del estudio fue determinar la rentabilidad de la producción de semilla en dos estaciones del año en pradera establecida de pasto buffel (*Cenchrus ciliaris* L) cv Zaragoza 115 bajo el efecto de fitohormonas y fertilización química en condiciones de riego en el norte de Coahuila. El trabajo se realizó en dos estaciones (abril-junio y agosto-octubre) en una pradera con 25 años de edad. En esta

<sup>1</sup> Agradecimientos al INIFAP por el financiamiento para el desarrollo del trabajo de investigación.



se estableció un diseño de bloques al azar con arreglo de parcelas divididas y cuatro repeticiones, para evaluar el efecto de cuatro fitohormonas: 1. Auxinas, 2. Giberelinas, 3: 1 + 2 y 4. Auxinas + Macro y Micronutrientes (MANyMIN) y cuatro concentraciones por fitohormona y MAN yMIN, incluida la dosis cero como testigo (0, 15, 30 y 45 mg l<sup>-1</sup>); en las variables de respuesta, número de panículas (NP ha<sup>-1</sup>), rendimiento de semilla cruda (RSC ha<sup>-1</sup>), porcentaje de semilla cruda desprendida (SCD), porcentaje de germinación de semilla cruda (% GSC) y la relación B/C. Los datos obtenidos se sometieron a análisis de varianza y comparación de medias (Tukey, P<0,05). Los resultados mostraron rentabilidad (Relación B/C de 1.41) en la producción de semilla en la primera estación con la aplicación de fitohormonas + macro y micronutrientes, al influir (P<0.05) en las variables de respuesta, logrando incrementar en 55.17 kg (125.38 a 180.55 kg ha<sup>-1</sup>) el RSC con la aplicación de 15 mg l<sup>-1</sup> de auxinas y 15 mg l<sup>-1</sup> de MANyMIN. Lo cual no ocurrió en la segunda estación del año, al obtener una relación B/C menor a 0.60, con rendimientos de 61,67 kg ha<sup>-1</sup>. Se concluye que la producción de semilla del pasto buffel Z115 en la estación de abril a junio en praderas con años de establecidas y con disponibilidad de riego, es técnica y económicamente rentable con la aplicación de auxinas y fertilización química.

**PALABRAS CLAVE:** Buffel Z115. Fitohormonas. Fertilización. Semilla. Rentabilidad.

## 1 INTRODUCCIÓN

En México, los agostaderos y áreas de pastizal de las zonas áridas y semiáridas del norte y el centro del País, representan cerca del 25% de los recursos naturales de la superficie nacional, base del sustento económica de los sistema de producción de carne bajo sistemas extensivos (INIFAP, 2008). La inadecuada planeación del pastoreo en ellos a través del tiempo y en años de sequías severas han acentuado su deterioro, presentando reducciones del 26% y 14% en cobertura vegetal y productividad, respectivamente en los últimos 50 años (SEMARNAP, 2015). Situación que ha ocasionado una baja rentabilidad en los ranchos deteriorados, la cual puede revertirse mediante la rehabilitación del 20 % de la superficie del rancho con siembras de pasto buffel con y sin financiamiento y apoyo gubernamental, al generar un rendimiento económico promedio anual de 25.7 veces superior al rancho deteriorado a partir de siete y ocho años (Ibarra *et al.*, 2007). La rehabilitación integral de éstos, demanda el uso de semilla de especies de pastos nativos e introducidos con adaptación probada al área objetivo (Terrazas, 2013; Volaire, 2018; Rajora *et al.*, 2020; Jurado *et al.*, 2021). En Coahuila existe un potencial de 10 millones de ha<sup>-1</sup> para la rehabilitación con especies nativas y pasto buffel (Martínez, 2000), requiriéndose para el caso de buffel, 3400 toneladas de semilla. Con la cuál no se puede disponer en el corto y mediano plazo y sobre todo del buffel Zaragoza 115, que en sus evaluaciones en la región mostro excelentes características productivas y adaptación a las condiciones agroclimáticas del estado (Osuna,1986). No obstante, la baja producción y disponibilidad de semilla de calidad de dicho pasto y de

otras especies forrajeras en México, por falta de programas de producción rentables ha limitado su adopción para contribuir a dicha demanda potencial (Hernández *et al.*, 2004; Quero *et al.*, 2007; Erazo *et al.*, 2022) al presentar pobre sincronización de floración y rápida dehiscencia de semilla, afectando el rendimiento y su rentabilidad (Hernández *et al.*, 2004). Problema abordado por investigadores a través de estudios de mejoramiento genético (Beltrán *et al.*, 2008; Terrazas, 2013; Erazo *et al.*, 2022; Gómez *et al.*, 2022) y prácticas agronómicas como, niveles de fertilización química, espaciamiento entre surcos y altura de corte (Quero *et al.*, 2007; Joaquín *et al.*, 2009; Kizima *et al.*, 2014). Así como también, mediante el uso de fitohormonas (Espinoza y Ortegón, 1993; Eguiarte y González, 2002; Joaquín *et al.*, 2006) con las cuales se reportan incrementos en el rendimiento de semilla de 20 a 236%. Atributo de las auxinas, al reducir la caída prematura de hojas, flores y frutos en cultivos en función de la dosis y etapa de aplicación (Vázquez y Yvanosky, 2018). Con base en lo anterior y ante la investigación limitada sobre el uso de fitohormonas en pastos. El objetivo del estudio fue determinar la rentabilidad de la producción de semilla en dos estaciones del año en pradera establecida de pasto buffel (*Cenchrus ciliaris* L.) cv Zaragoza 115 bajo el efecto de fitohormonas y fertilización química en condiciones en riego en el norte de Coahuila.

## 2 MATERIALES Y MÉTODOS

El estudio se realizó en dos estaciones (abril-Junio y agosto-octubre) del año de 2022 en el Sitio Experimental Zaragoza-INIFAP, ubicado en Zaragoza, Coahuila, a 28. 59754 latitud norte y -100. 90770 longitud oeste a 350 msnm; en un clima Bs0xh' (seco semi-cálido) con lluvias en verano y escasas a lo largo del año. Con temperatura media anual de 21.4° C, precipitación media anual de 375 mm con distribución bimodal. El suelo del área de estudio, se caracteriza por textura franco arcillosa, densidad aparente de 1.08 g/cm<sup>3</sup>, moderado contenido en materia orgánica (1.83 %) y sin problemas de salinidad; muy pobre en nitrógeno (N), medio en fósforo (P) y potasio (K), contenido bajo a medio en elementos menores; limitados en su disponibilidad por el pH alcalino (8.39) y muy alto contenido de carbonatos totales (68.1%) (Fertilab, 2022). Se utilizaron 900 m<sup>2</sup> de pradera de pasto buffel cv Zaragoza 115, establecida en 1997.

Se trazaron parcelas experimentales (PE) de 5 m<sup>2</sup> (2.5 m x 2.0 m) con parcela útil (PU) de 2 m<sup>2</sup> (1.0 m x 2.0 m), bajo un diseño experimental de bloques al azar con arreglo de parcelas divididas y cuatro repeticiones. Se evaluaron cuatro tratamientos de fitohormonas: 1: Auxinas, 2: Giberelinas, 3: 1 + 2 y 4: Auxinas + macro y micronutrientes, con cuatro dosis por fitohormona, incluido el testigo cero (0,15,30 y 45 ppm). Para la

fertilización foliar de macro y micronutrientes (MANyMIN), se uso un complemento foliar mediante una solución nutritiva a base de: N (66 g), P (49.5 g), K (35 g), Mg (150 mg), S (300 mg), Ca (150 mg), micronutrientes: B (225 mg), Cu (200 mg), Mn (225 mg), Fe (300 mg), Zn (450 mg), Mo (20 mg), Co (15 mg) en 300 a 400 litros agua ha<sup>-1</sup>.

Al inicio de cada estación del año, se uniformizó el pasto cortando este a 05 cm de la superficie del suelo. En cada una de las estaciones se fertilizó bajo la fórmula NPK (100-50-50), misma que se ajusto con base en los resultados del análisis de suelo. En total se dieron cuatro y dos riegos en la primer estación y segunda estación, con frecuencia de 13 y 18 días y laminas de agua por riego de 13 cm, respectivamente. Los tratamientos se aplicaron vía foliar al 50% de antesis de las plantas, por la mañana temprano (7:00 am). Se avaluó las variables, número de panículas (NP ha<sup>-1</sup>), rendimiento de semilla cruda (RSC kg ha<sup>-1</sup>), porcentaje de semilla cruda desprendida (SCD) porcentaje de germinación de semilla cruda (GSC) y la relación B/C.

El NP se determinó directamente cortando en forma manual con tijeras de podar todas las panículas presentes de panículas completas (PC) y panículas incompletas (PI), con semilla en punto de cosecha en cada unidad experimental en tres ocasiones durante el periodo del 08 al 18 de junio y en una ocasión (14 de octubre) en la segunda estación, mismas que se colocaron en bolsas de papel con el registro de fecha de cosecha, número de parcela y tratamiento y, número de panículas.

Posteriormente, se separó al azar de cada tratamiento, una muestra de cinco PC y una de cinco PI, ambas se colocaron en otra bolsa de papel con sus datos respectivos. Un mes después, se separó la semilla de cada muestra respectiva y se registré su peso en balcula digital con precisión de 0.001g, y con el peso promedio de éstas y el NP se determinó el RSC.

El porcentaje de SCD previo a la cosecha se estimó mediante la fórmula de kamal *et al* (2022) ajustada a las condiciones del presente estudio por carecer de equipo de mayor precisión, misma para la cual se peso la semilla de una muestra al azar de cinco PI y el peso promedio de éstas, se dividió entre el peso promedio de la semilla de una muestra de cinco PC, multiplicado por 100 y restado el valor obtenido de 100.

El porcentaje de GSC, se obtuvo mediante la semilla germinada de un total de 100 flósculos que se colocaron en cajas Petri, provistas éstas, con almohadillas de algodón y colocadas en una mesa de trabajo bajo condiciones ambientales (Temperaturas promedio de 39 ± 3 °C). El registro de la semilla germinada fue a través de conteos cada siete días por un periodo de 28 días. La semilla colocada en cada caja Petri, se tomó al azar de cada tratamiento con 11 meses de almacenamiento. Para estimar la relación

B/C, se empleo la formula  $B/C = \text{VAI (Valor actual de ingresos o beneficios descontados)} / \text{VACP (Valor actual de costos de producción asociados a la tecnología del proyecto)}$  (Ucañán, 2023), utilizando el registró de egresos, ingresos, rendimientos de semilla y precio de la semilla en el mercado regional de los tratamientos de fitohormonas, otros insumos y actividades y, se analizó solo el tratamiento sobresaliente en RSC con respecto al testigo, sin dejara afuera el manejo tradicional. A los datos obtenidos de la variables de respuesta, excepto a los de la relación B/C, se les realizó el análisis de varianza y pruebas de comparación de medias (Tukey $<0.05$ ) y pruebas de homogenización (en los casos de variables de datos registrados en porcentaje, se transformaron a arco seno  $\sqrt{\%}$ ). A los datos de germinación se realizó análisis de varianza de comparación de medias bajo un diseño completamente al azar). Se realizó un análisis de Pearson para estimar la correlación entre RSC y NP en las dos estaciones del año, mediante el programa estadístico SAS, versión 9.4. (SAS, 2004).

### 3 RESULTADOS Y DISCUSIÓN

#### 3.1 NÚMERO DE PANÍCULAS (NP)

El número de panículas (NP) durante la primera estación (PE) del año (abril-junio) se muestra en Tabla 1. En ésta se observa que el mayor NP ( $P < 0.05$ ) se obtuvo con la aplicación de 15 ppm de I.A de AIB + 0.5 L ha<sup>-1</sup> de macro y micronutrientes (T14) con un total de 1,785.0 mil panículas ha<sup>-1</sup>, superior e igual al T16 (AIB-45+MANyMIN- 45) y T13 (AIB-0+MANyMIN- 0) en 7.01 y 21.07%, equivalentes en 125,200 y 376,200 panículas ha<sup>-1</sup>, y superior al resto, donde el T12 produjo la menor cantidad.

En la segunda estación (SE) del año, el número de panículas (NP) (Tabla 1), no reporta efecto ( $P > 0.05$ ) entre tratamientos, con un una diferencia mínima significativa de 113.75 miles de panículas ha<sup>-1</sup> entre el tratamiento T15: (AIB-30+MANyMIN-30) con el mayor NP (328.75 miles ha<sup>-1</sup>) y el tratamiento T10 (AIB+AG<sub>3</sub>-15) con el menor NP (215.0 miles ha<sup>-1</sup>), respectivamente. En dicho rango el T15 y T14 superaron en 15.29 y 14.31% a T5 (AG<sub>3</sub>-0) y en 17.11 y 16.15% a T1 (AIB - 0), en forma respectiva. En esta SE al igual que en la PE del año, la fitohormona AIB+MAN y MIN bajo dosis de 30 y 15 ppm de I.A. (T14 y T16) reportaron los mejores resultados.

Los Incrementos obtenidos en la PE y SE del año, son inferiores al 48.72% reportado por Joaquín *et al* (2006) en pasto guinea (*Panicum máximum* Jcq) cv Tanzania con 6 mg kg<sup>-1</sup> I.A de la fitohormona esteroideal (cidef-4). La mejor respuesta de T14 y T16, posiblemente fue debido al contener en su composición, macro y micronutrientes que corrijan deficiencias en el suelo de estudio y su disponibilidad al ser bloqueados

por el alto pH (8.39) y los altos carbonatos (68.1%), indicados en los análisis de suelo (Fertilab, 2022).

Tabla 1. Comportamiento medio de panícula y rendimiento de semilla de buffel Z115 ante el efecto de fitohormonas en dos estaciones del año en el S.E. Zaragoza-CESAL-CIRNE-INIFAP.

TRATAMIENTO (Fitohormona- mg l <sup>-1</sup> )	Primera Estacion (PE) (abril-junio)		Segunda Estacion (SE) (agosto-octubre)	
	NP (Miles ha <sup>-1</sup> )	RSC (kg ha <sup>-1</sup> )	NP (Miles ha <sup>-1</sup> )	RSC (kg ha <sup>-1</sup> )
T14: AIB-15+MANyMIN-15	1,785.0 <sup>a</sup>	180.55 <sup>a</sup>	325.00 <sup>a</sup>	61.67 <sup>a</sup>
T16: AIB-45+MANyMIN-45	1,659.9 <sup>ba</sup>	174.08 <sup>ba</sup>	275.00 <sup>a</sup>	46.30 <sup>bac</sup>
T13: AIB-0+MANyMIN-0	1,408.8 <sup>bac</sup>	125.38 <sup>bac</sup>	237.50 <sup>a</sup>	49.86 <sup>bac</sup>
T15: AIB-30+MANyMIN-30	1,264.5 <sup>bdac</sup>	124.15 <sup>bac</sup>	328.75 <sup>a</sup>	46.91 <sup>bac</sup>
T1: AIB-0	1,228.8 <sup>bdc</sup>	102.23 <sup>bac</sup>	272.50 <sup>a</sup>	60.54 <sup>a</sup>
T9: AIB+AG <sub>3</sub> -0	1,152.5 <sup>bdc</sup>	123.35 <sup>bac</sup>	231.50 <sup>a</sup>	41.02 <sup>bac</sup>
T8: AG <sub>3</sub> -45	1,126.3 <sup>bdc</sup>	92.33 <sup>c</sup>	312.00 <sup>a</sup>	46.30 <sup>bac</sup>
T5: AG <sub>3</sub> -0	1,100.0 <sup>dc</sup>	91.70 <sup>c</sup>	278.50 <sup>a</sup>	37.52 <sup>bac</sup>
T4: AIB-45	1,090.0 <sup>dc</sup>	86.17 <sup>c</sup>	297.50 <sup>a</sup>	40.65 <sup>bac</sup>
T3: AIB-30	1,078.8 <sup>dc</sup>	68.60 <sup>c</sup>	317.50 <sup>a</sup>	34.12 <sup>c</sup>
T10: AIB+AG <sub>3</sub> -15	1,076.3 <sup>dc</sup>	108.50 <sup>bac</sup>	215.00 <sup>a</sup>	28.46 <sup>c</sup>
T7: AG <sub>3</sub> -30	1,048.8 <sup>dc</sup>	81.28 <sup>c</sup>	242.50 <sup>a</sup>	41.07 <sup>bac</sup>
T2: AIB-15	1,028.8 <sup>dc</sup>	94.73 <sup>bc</sup>	322.50 <sup>a</sup>	40.33 <sup>bac</sup>
T11: AIB+AG <sub>3</sub> -30	1,026.3 <sup>dc</sup>	89.50 <sup>c</sup>	245.00 <sup>a</sup>	44.47 <sup>bac</sup>
T6: AG <sub>3</sub> -15	942.5 <sup>dc</sup>	83.25 <sup>c</sup>	251.50 <sup>a</sup>	46.30 <sup>bac</sup>
T12: AIB+AG <sub>3</sub> -45	847.5 <sup>d</sup>	98.95 <sup>bc</sup>	258.50 <sup>a</sup>	41.67 <sup>bac</sup>
DMS	553.5	81.48	182.00	26.13

**DMS:** Diferencia mínima significativa. Para cada columna, valores con diferente literal son estadísticamente diferentes (P<0.05). **NP:** Número de panículas; **RSC:** Rendimiento de semilla cruda.

**Nota: Fitohormonas y fertilización foliar con macro y macronutrientes utilizados:**

- Auxinas:** Se uso el AIB: Ácido Indol butírico vía el producto comercial: Radix® 10000 PS.
- Giberelinas:** Se uso AG<sub>3</sub> a través del producto comercial: Biogib® 10PS.
- Macronutrientes y Micronutrientes (MANyMIN):** Un complemento de fertilización foliar mediante una solución nutritiva a base de: N (66 g), P (49.5 g), K (35 g), Mg (150 mg), S (300 mg), Ca (150 mg), micronutrientes: B (225 mg), Cu (200 mg), Mn (225 mg), Fe (300 mg), Zn (450 mg), Mo (20 mg), Co (15 mg) en 300 a 400 litros de agua ha<sup>-1</sup>.

El NP obtenido en el presente trabajo con T14, son inferiores a los reportados por Eguiárte y González (2002), quienes obtuvieron en promedio 2,124.0 panículas (Miles ha<sup>-1</sup>), con un rango de 1,976.6 a 2,344.4 con la aplicación del inductor floral AGR® al trabajar por tres años en Jalisco, con inductores florales en una pradera recién establecida con buffel cv Biloela. La diferencia puede ser debida a la diferencia en la edad de ésta al

momento del estudio, con respecto a la edad de 25 años en la pradera de buffel Z 115 y que el NP corresponde a una de dos estaciones en el año.

El NP obtenido en la SE del año con T14 fue 81.79 % menor que en la PE, al obtener solo una pizca contra tres pizcas en la primera. La diferencia puede ser debido al retraso de 20 días en el inicio del trabajo experimental y al fotoperiodo al inicio y durante la SE del año el cual fue de 12 y 11 horas, los cuales muy posiblemente redujeron el número de espigas de acuerdo al estudio realizado por Evers *et al* (1969), quienes observaron dicho efecto en genotipos de buffel cuando el fotoperiodo se redujo de 12 horas.

### 3.2 RENDIMIENTO DE SEMILLA CRUDA (RSC)

Al igual que en la variable NP durante la PE en el año, el RSC fue afectado ( $P < 0.05$ ) por los tratamientos (Tabla 1). El mayor rendimiento lo registró T14 con 180.55 kg ha<sup>-1</sup>, superior e igual a T16 y T13 con incrementos del 3.72 y 44.00 % (6.47 y 55.17 kg ha<sup>-1</sup>), respectivamente.

En la SE del año, el RSC fue afectado ( $P < 0.05$ ) por los tratamientos (Tabla 1), los mayores rendimientos los registraron el T14 y el testigo T1 con 61.67 y 60.54 kg ha<sup>-1</sup>, iguales entre y superiores a 12 tratamientos con rendimientos de 49.80 a 40.33 kg ha<sup>-1</sup>. El incremento en T14 con respecto al testigo T1, fue del orden del 1.83 %.

El incremento en el rendimiento del 44% en la PE del año con respecto al testigo, es 34% mayor al reportado por Joaquín *et al* (2006) con aplicación de fitohormona esteroideal (cidef-4) a dosis de 6 mg kg<sup>-1</sup> de I.A. Pero inferior al incremento de 50% obtenidos por Eguiarte y González (2002) en buffel Biloela con el inductor floral AGR en condiciones de temporal en Jalisco, en pradera recién establecida y en tres cosechas por año.

Los rendimientos totales de la PE y SE del año, superan los 60 kg ha<sup>-1</sup>, reportados en buffel Zaragoza 115 en tres pizcas en Zaragoza, Coahuila, por Cuellar y Hernández (1989) con fertilización 100-50-50 y riego; a los 78 kg ha<sup>-1</sup>, por Lara (1989), en dos pizcas en la misma variedad en Zaragoza, Coahuila y en condiciones de riego y fertilización (100-50-00), a los 89 kg ha<sup>-1</sup> por Martínez (1996) y similares (242 kg ha<sup>-1</sup> vs 249 kg ha<sup>-1</sup>) a los de Briones (1991) con buffel común en tres cosecha en el año.

El incremento en la SE del año con el T14 (AIB-15+MANYMIN-15) con respecto al testigo T1 (AIB-0), fue del orden del 1.83%. Incremento muy inferior al 44% obtenido en la PE del año, al 34 y 50 % logrado por Joaquín *et al* (2006), Eguiarte y González (2002), respectivamente.

La respuesta positiva en el RSC con respecto al testigo T1 (AIB-0) en la PE del año, pudo deberse al aumento del NP, componente de rendimiento que se asocia en alto grado

a dicha variable con valor de 0.8966, y sin asociación en la SE con valor de 0.2732 (Tabla 2). El primero, es similar al valor de 0.8342, reportado por Joaquín *et al* (2006) en pasto guinea, quienes al correlacionar varias componentes de rendimiento con rendimiento de semilla pura viba, el NP fue el de mayor asociación. En cambio, la baja asociación en la SE del año, puede deber al fotoperiodo, radiación y otros factores ambientales, mismo que de acuerdo a Evers *et al* (1969) influye positivamente en los componentes de rendimiento de semilla.

Tabla 2. Coeficientes de correlación entre rendimiento de semilla cruda (RSC) y número de panículas (NP) en dos estaciones del año en pasto buffel Z115. S.E. Zaragoza-C.E. Saltillo-CIRNE -INIFAP.

Estación del año/variable	RSC (kg ha <sup>-1</sup> )
<b>Primera estación:</b>	
Número de panículas	0.8966**
<b>Ignificativo</b>	
Número de panículas	0.2732 NS
**.:Significativo (p<0.0001): NS: No significativo.	

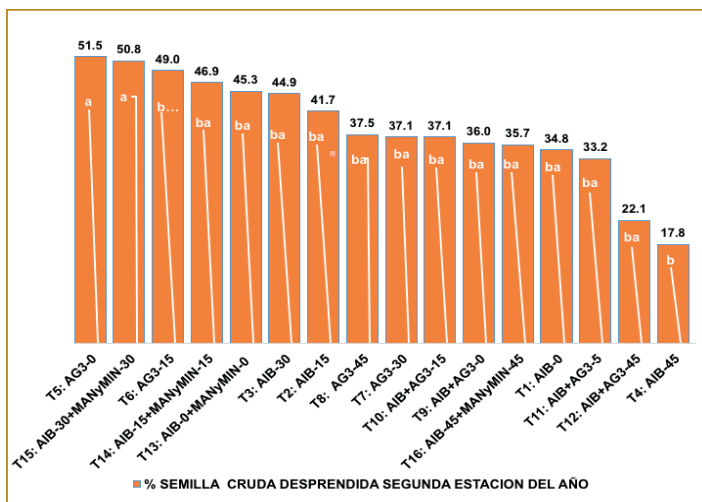
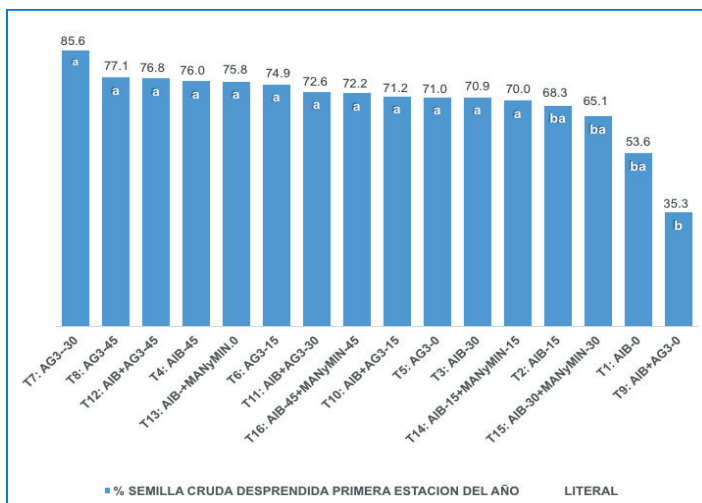
La diferencias en la respuesta de la especie a los insumos, también es debido a las condiciones ambientales, temperatura, pH del suelo y fotoperiodo estacional, las cuales inciden en los rendimientos de semilla al afectar el número de espigas por planta, número y peso de flósculos por espiga y largo de espigas (Conde *et al.*, 2011; Eguiarte y González, 2002, Joaquín *et al.*, 2006).

### 3.3 SEMILLA CRUDA DESPRENDIDA (SCD)

En las Figuras 1a y 1b se muestra la comparación de medias del por ciento de SCD bajo el efecto de fitohormonas en diferentes dosis durante la PE y SE del año. Los resultados en la PE (Figura 1a) muestran el efecto (P<0.05) de los tratamientos, donde 12 tratamientos (T7, T8,T12, T4, T13, T6, T11,T16, T10, T15, T13 y T4,) con un rango de 85.6 a 70.0% presentaron los mayor valores de SCD entre sí, con diferencia mínima de 15.6 % y superiores e iguales a T2 (AIB-15) , T15 (AIB-30+MANYMIN-30) y T1 (AIB-0), con valores de 68.3, 65.1 y 53.6% respectivamente, y superiores a T9, quien reportó la menor SCD (35.3%).

En la SE del año (Figura 1b), el efecto (p<0.05) de los tratamientos T5 (AG<sub>3</sub> -0) y T15 (AIB-30+MANYMIN-30) con valores de 51.5 y 50.8 % de SCD, fueron iguales entre sí, y superiores a 12 tratamientos (T6, T14, T13, T3, T2, T8, T7, T10, T9, T16, T1, T11, T12,) en un rango de 49.0 a 22.1 %, respectivamente, y estos, superiores a T4 (AIB-45), quien reportó la menor SCD (17.8%).

Figura 1a y 1b. Comparación de medias de semilla cruda desprendida (%) del pasto buffel Z115 ante tratamientos de fitohormonas y dosis de 0,15,30 y 45 mg l-1 de I.A en dos estaciones del año de 2022 (1a: abril-junio y 1b: agosto-octubre). (Literales diferentes en las columnas indican diferencia P<0.05) entre tratamientos.



Los valores de SCD se pueden atribuir al efecto del tratamiento de la fitohormona, vientos moderados acontecidos durante la etapa de maduración de la semilla y, al período entre cosechas durante la PE de año. La semilla desprendida es la semilla potencial producida por espiga que no se pudo cosechar y que podría rescatarse mediante pizcas cada tres días.

El tratamiento de mayor RSC ha<sup>-1</sup> T14 (AIB-15+MANyMIN-15) en la PE (Tabla 1) reportó 70% de desprendimiento de semilla (Figura 1a), ubicado dentro de los cinco tratamientos con menor valor, lo cuál respalda el mayor rendimiento de semilla. El valor obtenido de SCD con T14, superó al 50.9 % reportado por Joaquín *et al* (2009)



en pasto guinea (*Panicum maximum* cv Tanzania) bajo la dosis de fertilización de 100 kg N ha<sup>-1</sup> y al 34 % reportado por Joaquín *et al* (2006) en el mismo pasto con la aplicación de 6 mg kg<sup>-1</sup> de I.A de esteroideal (cidef-4) al inicio de antesis. Valores que están en el rango de 13 a 56 % en cebada, reportado por Kamal *et al* (2022); no así, el 70% obtenido en el presente estudio con el T14 con el mayor RSC, el cuál fue superior en 20 % al valor más alto (56%) del rango, pero que fue el quinto con menor desprendimiento de semilla.

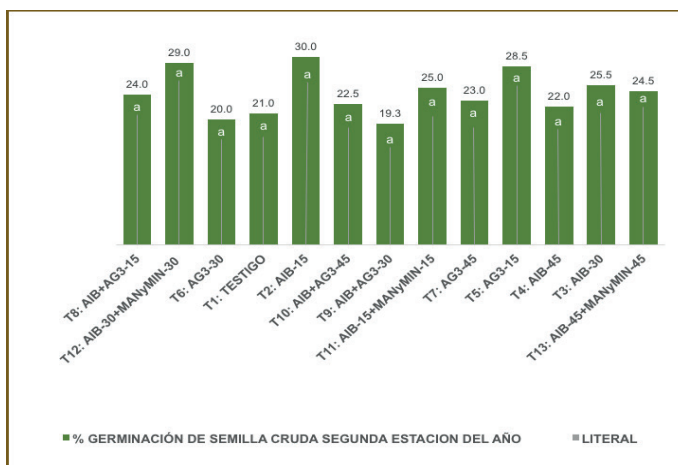
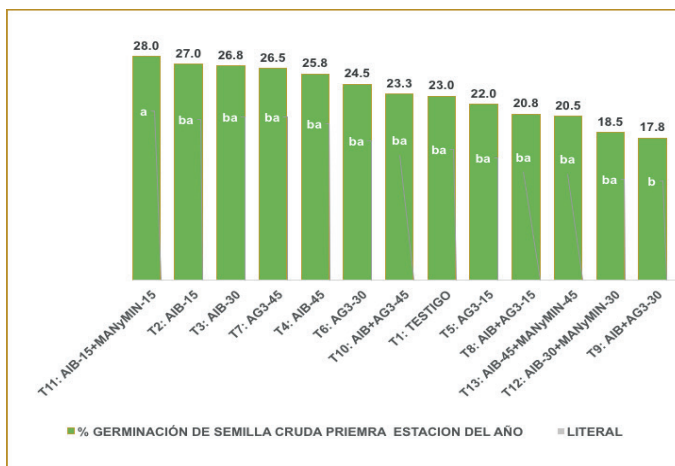
En cambio, en la SE del año, los valores de SCD en todos los tratamientos con rango entre el menor y mayor (17.8 a 51.5 %), están dentro del rango (13 a 56%) observado por Kamal *et al* (2022). A excepción del T4 (AIB-45), los demás tratamientos con valores de SCD son similares al 50.19 % reportados en buffel (Eguiarte y González, 2002), al 50.9 % reportados por Joaquín *et al* (2001) con 100 kg N ha<sup>-1</sup> en pasto guinea y al 32.6% observado por Joaquín *et al* (2006) con la fitohormona esteroideal a dosis de 6 mg kg<sup>-1</sup> en el mismo pasto.

### 3.4 GERMINACIÓN DE SEMILLA CRUDA (GSC)

El valor medio del por ciento de GSC, bajo los tratamientos de evaluados en la PE del año, se muestra en la Figura 2a. El mayor valor (28 %) se obtuvo con T11 (AIB-15 +MANyMIN-15) el cual fue superior e igual ( $P<0.05$ ) en 17.86 % al T1 (Testigo: AIB-0) que obtuvo 23 % de germinación.

En la SE del año, los valores medios del por ciento de la GSC (Figura 2b), no se afectaron ( $P<0.05$ ) por los tratamientos, siendo iguales entre sí con diferencia mínima significativa de 10.7 %. El mayor valor (30%) se obtuvo con T2 (AIB-15) el cual superó en 30.0 % al T1 (Testigo: AIB-0) con 21.0 %. El T11 (AIB-15+MANyMIN-15) con 25 % de germinación, y que registro el mayor RSC de 61.67 kg ha<sup>-1</sup> (Tabla 1), también superó al testigo (T1: AIB-0) en 16 %.

Figura 2a, 2b. Comparación de medias de germinación de semilla cruda (%) del pasto buffel bajo el efecto de tratamientos de fitohormonas y dosis de 0,15,30 y 45 mg l<sup>-1</sup> de I.A en dos estaciones del año de 2022 (2a: abril-junio y 2b: agosto-octubre). (Literales diferentes en las columnas indican diferencia P<0.05) entre tratamientos.



Valores similares (28.2%) en *Cenchrus ciliaris* L, en semilla con cinco meses de almacenamiento y pruebas bajo condiciones de laboratorio, reportaron Palma *et al* (2000), quienes mencionan que además del tiempo de almacenamiento, la humedad relativa y la temperatura, entre las causas que influyen significativamente en la germinación, está el tipo de envase para el almacenamiento al afectar la calidad fisiológica de la semilla.

En la PE del año, el T11 (AIB-15+MANyMIN-15), superó en 39.29.% el valor máximo (17%) reportado por Sáenz *et al* (2015) en buffel en Aldama, Chihuahua y, en la SE, el T2 y T11, lo superaron en 43.33 y 32.00 %. No así el 65.5% reportados por Lara (1998), en ensayos de germinación de semilla (flósculos) de buffel Zaragoza 115 con seis meses de almacenamiento y periodo de prueba de 13 días con tratamiento de calor y frío a la semilla previo al ensayo.

La diferencia a favor de los valores reportados por Lara (1998), se debe a las buenas condiciones de almacenamiento de la semilla y al equipo disponible para proveer tratamiento de frío y calor a la misma y, usar semilla de pradera de reciente establecimiento con separación entre plantas mayor a 50 cm. En el presente trabajo se utilizó semilla de una parralera de 25 años de edad, con espaciado entre plantas menor a 25 cm, condiciones que afectan la calidad de la semilla, como lo observaron Kizima *et al* (2014) en estudio en parraleras viejas y con distancia entre plantas menores a 25 cm y, muy posible a la acumulación de inhibidores de la semilla al usar cajas petri (Ayerza, 1981) que no permite que la especie exprese su máximo potencial germinativo.

### 3.5 RELACIÓN COSTO-BENEFICIO (B/C)

Los conceptos considerados para el análisis de la relación B/C de la producción de semilla de pasto buffel Zaragoza en pradera con 25 años de establecida bajo las diferentes tecnologías, como el **manejo tradicional** (sin fertilización NPK 100-50-50 y AIB-15+MANyMIN-15), el **testigo experimental** (con NPK 100-50-50 y sin AIB-15+MANyMIN-15), el **componente tecnológico** (AIB-15+MANyMIN-15), el paquete tecnológico y/o **tecnología INIFAP** (con NPK 100-50-50 y AIB-15+MANyMIN-15), durante la PE y SE del año 2022, se aprecian en Tabla 3.

Tabla 3. Relación costo-beneficio (B/C) de la producción de semilla de pasto buffel Z115 con manejo tradicional y el tratamiento de fitohormona y fertilización con el mayor rendimiento de semilla cruda (AIB-15+MANyMIN-15) en la primera estación (PE) y segunda estación (SE) del año (abril-junio y agosto-octubre 2022). S.E-Zaragoza-C.E. Saltillo-CIRNE-INIFAP.

Concepto	Tecnología							
	Manejo Tradicional		*Testigo Experimental		**Componente AIB-15+MANyMIN-15		*Tecnología INIFAP (Nueva)	
	PE	SE	PE	SE	PE	SE	PE	SE
<b>1. C. P. (\$ ha<sup>-1</sup>)</b>	4,100	4,230	13,505	13,168	545	545	14,992	14,213
<b>2. R.S. (Kg ha<sup>-1</sup>)</b>	15	15	125.38	60.5	180.55	1.2	55.17	61.7
<b>3. R.P. (ha<sup>-1</sup>)</b>	--	4	--	18	--	--	--	20
<b>4. I.B. (\$ ha<sup>-1</sup>)</b>	3,000	4,800	25,076	20,208	11,000	240	36,110	21,334
Semilla	3,000	3,000	25,076	12,108	11,000	240	36,110	12,334
Rollo pasto.	--	1,800	--	8,100	--	--	--	9,000
<b>5. I.N. (\$ ha<sup>-1</sup>)</b>	-1,100	570	11,571	7,040	10,454	-305	21,118	7,121
<b>6. B/C</b>	-0,27	0.13	0.86	0.53	19.18	-0.56	1.41	0.50

**Nota:** CP: Costos de producción= Actividades + insumos y servicios; IBT: Ingreso bruto total = kg de semilla x \$200 + Número de Rollos x \$450; IN: Ingreso Neto= IBT-IN; B/C= IN/CP.  
 \* Los insumos y servicios representan entre el 52 al 84% de los costos de producción.  
 \*\* Insumos representan el 36% de los costos de producción ( \$ 195.0= \$ 50.0, 62.5 y 82.5 por concepto del uso de 15 mg l<sup>-1</sup>, 0.5, 0.25 y 0.5 L ha<sup>-1</sup> de AIB+MANyMIN, Acidificante; pH -Fase y Adeherente, respectivamente) + \$350.0 por concepto de un jornal para la aplicación de productos)

La incorporación del **componente tecnológico AIB-15+MANyMIN-15** (15 ppm I.A de auxina (AIB) + 0.5 L ha<sup>-1</sup> de MANyMIN) en la PE al paquete tecnológico (**Tecnología INIFAP**), que incluye la fórmula de fertilización NPK100-50-50 y AIB-15+MANyMIN-15, reportó el mayor rendimiento de semilla cruda (180.55 kg ha<sup>-1</sup>), el cuál superó en 55.17 kg ha<sup>-1</sup>, el rendimiento de 125.38 kg ha<sup>-1</sup> del **Testigo experimental** (con solo NPK 100-50-50). Hecho que no aconteció en la SE del año, donde el mayor rendimiento (61.7 kg ha<sup>-1</sup>) en Tecnología INIFAP al incorporar el AIB-15+MANyMIN-15, supero en 1.2 kg ha<sup>-1</sup>, el rendimiento de 60.5 kg ha<sup>-1</sup>. del testigo experimental.

Los costos generados con la aplicación del componente AIB-15+MANyMIN-15 en la PE del año para la obtención de dicho incremento en el rendimiento de semilla fue de \$ 545.00 (Por el uso de 15 mg l<sup>-1</sup>+15 mg l<sup>-1</sup> y , 0.250 y 0.500 L ha<sup>-1</sup> de AIB+MANyMIN, acidificante y adherente y, un jornal para su aplicación) y, al deducirle el ingreso por concepto de venta de los 55.2 kg de semilla a precio regional de \$ 200.00 kg<sup>-1</sup>, generó un ingreso bruto de \$ 11,040.00 y restando a este, el costo del componente AIB-15+MANyMIN-15 de \$545.00, arrojó un ingreso neto de \$ 10,495.00 y una relación B/C de 19.2:1, positiva y muy superior a la relación de la SE del año de -0.56.

Suceso similar ocurrió en la PE, al analizar los costos del paquete tecnológico (Tecnología INIFAP) que propiciar una relación positiva B/C = 1.41, con respecto a la B/C = 0.50 en la SE donde no se recupera la unidad invertida. Ésta misma situación aconteció en la B/C, con el testigo experimental en la PE y SE, con valores de 0.86 y 0.53; siendo más severa esta relación bajo el manejo tradicional con B/C de -0.27 y 0.13 en la PE y SE del año, respectivamente. Esta última con rendimientos de 15 kg ha<sup>-1</sup>, sin la aplicación de NPK 100-50-50 y AIB-15+MANyMIN-15) (Tabla 3).

El beneficio que se obtuvo en la PE del año (abril-junio) fue superior al beneficio logrado por Kizima *et al* (2014) de 0.59 pesos por unidad invertida en buffel cv Biloela, con rendimientos de 78.6 kg ha<sup>-1</sup> y 13.2 ton ha<sup>-1</sup> de forraje seco, con la dosis de fertilización 60 kg N y 30 kg P ha<sup>-1</sup>, en la estación húmeda con irrigación en el primer año de establecimiento, debido a los costos por concepto de riegos y materiales e insumos para el establecimiento.

La mejor relación B/C obtenida en la SE del año (25 de agosto al 14 de octubre), fue de 0.50 con la tecnología INIFAP, similar a 0.59 que obtuvo Kizima (2014), las cuales no son rentables. Debido a los bajos rendimientos de semilla (61.7 kg ha<sup>-1</sup>) y al fotoperiodo menor a 12 horas, en septiembre. Rendimientos que se podría mejorar y obtener relaciones mayores a 1.0 mediante evaluaciones donde se incorporé micorrizas y dosis de fertilización química menores a la fórmula recomendada de 100-50-50, ya que ésta

representa entre el 59 al 84% de los costos de producción. A su vez continuar con la evaluación de AIB+MANyMIN en diferentes etapas del cultivo e iniciar la segunda estación (SE) a partir del 15 de agosto para aprovechar el fotoperiodo de 12 horas luz en el mes de septiembre, mismo que de acuerdo a Evers *et al* (1969) influye positivamente en los componentes de rendimiento de semilla.

#### 4 CONCLUSIONES

Es técnicamente viable la rentabilidad de la producción de semilla de buffel Z115 en la PE del año, en praderas con varios años de establecimiento bajo manejo tradicional y en las que están bajo manejo de fertilización NPK 100-50-50; con la aplicación de la Tecnología INIFAP (NPK 100-50-50 y AIB-15+MANyMIN-15) en el primer caso, y mediante el componente tecnológico AIB-15+MANyMIN-15, en el segundo, al obtener una relación B/C de 1.41 por unidad invertida, con RSC de 180.55 kg ha<sup>-1</sup>; y al incrementar en 55.17 kg ha<sup>-1</sup> los 125.38 kg ha<sup>-1</sup> (obtenidos en praderas establecidas con NPK 100-50-50), con solo aplicar el componente tecnológico AIB-15+MANyMIN-15 (15 mg l<sup>-1</sup> I.A de AIB+15 mg l<sup>-1</sup> de MANyMIN en 300 a 400 L ha<sup>-1</sup>), generó una relación B/C de 19.18 por unidad invertida, sin incluir los ingresos por concepto de venta de forraje.

La rentabilidad en la SE del año, con RSC de 61.67 kg ha<sup>-1</sup>, no es viable con la aplicación del componente tecnológico y tampoco con la inclusión de dicho componente en todo el paquete tecnológico (Tecnología INIFAP), al reportar valores de B/C menores de 0.60.

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# CAPÍTULO 7

## A IMPORTÂNCIA CRESCENTE DOS PRODUTOS AGRÍCOLAS E AGROALIMENTARES LOCAIS: OS EFEITOS DA PANDEMIA COVID-19 NOS CIRCUITOS CURTOS DE PROXIMIDADE<sup>1</sup>

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**RESUMO:** A crescente oferta de produtos agrícolas e agroalimentares, largamente disponíveis nas grandes superfícies de distribuição (hiper e supermercados), a que se junta as mudanças operadas nos próprios ritmos de vida das pessoas, tem alterado drasticamente os hábitos de alimentação, sobretudo dos meios urbanos. Questões como a origem, a qualidade e segurança dos alimentos, são agora mais do que noutras alturas, inicialmente desconsideradas. Neste contexto, as produções locais e os circuitos curtos de proximidade perspetivam-se como uma alternativa eficaz de distribuição de produtos alimentares. Por outro lado, a

pandemia provocada pela COVID-19 veio apelar, mais do que em outras alturas, à necessidade de ser enfatizado o que é produzido e distribuído localmente. Para além dos benefícios económicos para os próprios produtores e benefícios ambientais para o meio ambiente, salientam-se ainda os benefícios sociais decorrentes desta prática, já que se fomenta a confiança entre produtores e consumidores. Daí que com base numa investigação desenvolvida em concelhos pertencentes à Região Viseu Dão Lafões, no Centro de Portugal, o objetivo deste trabalho é o de investigar o impacto da pandemia provocada pela COVID 19 ao nível os circuitos curtos de proximidade. Os resultados obtidos sugerem que a pandemia provocada pela COVID 19 consciencializou para a importância das produções locais e das temáticas relativas à qualidade dos produtos.

**PALAVRAS-CHAVE:** COVID-19. Produção Local. Circuitos curtos de proximidade. Segurança alimentar.

THE GROWING IMPORTANCE OF LOCAL AGRICULTURAL AND AGRIFOOD PRODUCTS: THE EFFECTS OF PANDEMIC COVID-19 ON THE SHORT FOOD SUPPLY CHAINS

**ABSTRACT:** The growing offer of agricultural and agrifood products, widely available in large distribution areas (hypermarkets and supermarkets), in addition to the changes

<sup>1</sup> Este trabalho foi parcialmente apresentado no 27 th APDR Congress Sustainable Management of the Sea for Sustainable Regional Development, encontrando-se uma versão preliminar nas atas da conferência: Pato, L. (2020). A (crescente) ênfase nos produtos agrícolas e agroalimentares produzidos localmente. os efeitos da pandemia covid 19 nos circuitos curtos de proximidade. In proceedings of 27 th APDR Congress Sustainable management of the Sea for sustainable Regional Development (322-326). Agra do Heroísmo, Terceira, Açores.



made in people's own rhythms of life, has drastically altered their eating habits, especially in urban areas. Issues such as origin, quality and safety of food, are now more than in other times, initially disregarded. In this context, local productions and short proximity circuits are seen as an effective alternative for the distribution of food products. On the other hand, the pandemic caused by COVID-19 came to appeal, more than at other times, to the need to emphasize what is produced and distributed locally. In addition to the economic benefits for the producers themselves and environmental benefits, it is highlighted social benefits, since trust between producers and consumers is fostered. Hence, based on an investigation carried out in some municipalities of Viseu Dão Lafões Region, the purpose of this work is to investigate the impact of the pandemic caused by COVID 19 at the level of short supply chains. The results suggest that the pandemic COVID-19 made aware of the importance of local productions and issues concerning food quality.

**KEYWORDS:** Covid-19. Local Production. Short-food supply chains. Food security.

## 1 INTRODUÇÃO

A melhoria da tecnologia e disponibilidade de fatores de produção tem permitido aumentar a produção de produtos agrícolas e agroalimentares de forma exponencial. Mercê deste aumento de produção e dos acordos comerciais estabelecidos internacionalmente, as importações e exportações de produtos entre diferentes países, nomeadamente de países pertencentes à União Europeia, tem aumentado igualmente de forma significativa (EC, 2017).

Assim, a crescente oferta de produtos agrícolas e agroalimentares, largamente disponíveis nas grandes superfícies de distribuição (hiper e supermercados), a que se junta as mudanças operadas nos próprios ritmos de vida das pessoas, tem alterado drasticamente os seus hábitos de alimentação (Cappelli & Cini, 2020), sobretudo dos meios urbanos. Questões como a origem, a qualidade e segurança dos alimentos, são agora mais do que noutras alturas, relegadas para segundo plano.

A pandemia provocada pela COVID 19, veio, no entanto, a provocar alterações profundas quer no abastecimento quer na procura de produtos agrícolas. Por um lado, do lado da oferta, devido ao encerramento das fronteiras, o comércio internacional foi inicialmente interrompido no auge da primeira vaga do surto pandémico. Embora tivessem posteriormente sido definidos protocolos de segurança para evitar a propagação do vírus e as fronteiras tenham reaberto, esta pode ser uma situação temporária, dependendo do que os países estão a fazer para evitar a propagação do vírus (Siche, 2020). Por outro lado, a procura de produtos agrícolas tem diminuído (embora que de forma ainda ténue) devido à redução da capacidade para as pessoas gastarem dinheiro e à incerteza gerada nas mesmas por um produto muitas vezes desconhecido (Siche, 2020).

É neste contexto que as produções agrícolas locais e as cadeias curtas alimentares se perspetivam como uma ferramenta interessante a considerar (Barberaa & Joselle, 2016), tanto mais que a pandemia provocada pela COVID-19 veio apelar, mais do que em outras alturas, à necessidade de ser enfatizado o que é produzido e distribuído localmente (Cappelli & Cini, 2020).

Para além dos benefícios económicos para os próprios produtores agrícolas, muitos deles numa situação vulnerável, salientam-se ainda os benefícios ambientais para o território, os benefícios socioculturais (Raftowicz, Kalisiak-Medelska, & Struś, 2020; Tibério, 2013) e o contributo para a saúde e bem estar das pessoas, cada vez mais importante nos dias de hoje. Daí que a pergunta de partida nesta investigação seja:

Qual o efeito da pandemia COVID-19 ao nível dos circuitos curtos de proximidade?

Partindo duma investigação exploratória em alguns concelhos da Região Viseu Dão Lafões (RVDL), o objetivo deste trabalho é pois o de analisar os efeitos que a pandemia provocada pelo COVID-19 trouxe ao nível do consumo de produtos agrícolas e agroalimentares locais. Para tal recorreu-se a métodos de investigação qualitativa, com destaque para a realização de entrevistas semiestruturadas a produtores e distribuidores de produtos agrícolas e agroalimentares. As entrevistas foram gravadas, transcritas e sujeitas à análise de conteúdo.

Os resultados preliminares indicam que a pandemia COVID-19 consciencializou para as temáticas da qualidade, segurança e origem dos produtos, sendo que se perspetiva uma procura crescente por parte de produtos produzidos localmente e como tal dos circuitos curtos de proximidade de produtos agrícolas e agroalimentares.

Não obstante a pandemia esteja ultrapassada, é importante considerar que somos vulneráveis a situações como a focada neste trabalho, pelo que em direção à segurança alimentar e proteção das pequenas explorações agrícolas, argumentamos a importância das produções locais.

## **2 PRODUÇÕES LOCAIS E CADEIAS CURTAS DE ABASTECIMENTO: REGRESSO AO PASSADO OU CAMINHO PARA O FUTURO**

Por um lado a modernização das estruturas de transformação e distribuição, bem como a inovação ao nível dos produtos e dos processos, contribuiu para uma maior diversidade e qualidade da oferta de produtos alimentares à escala nacional e para o reforço da acessibilidade dos consumidores a esses mesmos produtos (Duarte, 2013). Por outro lado, as alterações sociodemográficas da população em geral, como o ténue envelhecimento da população, a redução do agregado médio das famílias, o sedentarismo

do trabalho, a redução do tempo para comer e o aumento da população urbana que caracterizam, embora com expressão diversa, os países desenvolvidos nas últimas décadas (Duarte, 2013), tem contribuído para o aumento da procura por produtos de conveniência (congelados, enlatados, refeições pré-preparadas, saladas prontas a usar, etc.), disponíveis nas grandes superfícies de distribuição alimentar ou então para que grande parte da alimentação se faça a partir das cadeias de *fast-food*.

No entanto as notícias e investigações que tem sido divulgadas a propósito da alimentação feita a partir de alimentos processados e com ingredientes industriais que afetam gravemente a saúde colocam em causa este tipo de alimentação. Por exemplo Srour et al. (2019) observam que o alto consumo de alimentos processados está associado a riscos cardiovasculares, a enfartes cardíacos e doenças cerebrovasculares. A investigação de Hall et al. (2019) alerta também para que o excesso de dietas ultra-processadas contribuam para a ingestão de calorias e para o excesso de peso.

É neste contexto que desde há alguns anos a esta parte, algumas referências têm sido feitas sobre um possível regresso ao passado, no sentido de uma deslocação da procura alimentar para bens mais básicos, no seu estado mais natural e de menor valor acrescentando, contrariando assim as ofertas padronizadas da indústria alimentar (Duarte, 2013). Engloba-se ainda neste regresso ao passado, a procura por produtos biológicos, oriundos de uma agricultura menos intensiva (Nagy & Dabija, 2020) e a procura de produtos que combinam características tradicionais, locais e regionais (Fernández-Ferrín, Calvo-Turrientes, Bande, Artaraz-Miñón, & Galán-Ladero, 2018; Pieniak, Verbeke, Vanhonacker, Guerrero, & Hersleth, 2009), associando assim o produto ao território e promovendo o contacto direto entre produtos e consumidores (Duarte, 2013). Este regresso ao passado e a procura de produtos locais, mais naturais e menos processados, parece ganhar impulso em situações de crises e inseguranças alimentares (Wilkinson, 2011).

Embora transversais, estas tendências manifestam-se em graus diferentes consoante os países, e, num mesmo país, entre diferentes segmentos de consumidores (Duarte, 2013). Prevê-se que os consumidores que dão mais importância a este tipo de produtos sejam pessoas preocupadas com a sustentabilidade ambiental e com os produtos locais e ao mesmo com a sua saúde e bem-estar.

Esta tendência de procura de produtos tradicionais/locais/regionais, mais naturais e/ou proveniente de uma agricultura biológica, apresentam-se assim como “janelas de oportunidades” que começam a ser também exploradas pelos micro e pequeno produtores agrícolas. Evidência disso é a legislação existente tendo em vista o enquadramento de estratégias de desenvolvimento local. Por exemplo em Portugal a

portaria nº 152/2016 de 25 de maio (artigo 27) refere que os objetivos das produções locais e cadeias curtas são:

- a) promover o contacto direto entre o produtor e o consumidor, contribuindo para o escoamento da produção local, a preservação dos produtos e especialidades locais, a diminuição do desperdício alimentar, a melhoria da dieta alimentar através do acesso a produtos da época, frescos e de qualidade, bem como para o fomento da confiança entre produtor e consumidor;
- b) incentivar práticas agrícolas menos intensivas e ambientalmente sustentáveis, contribuindo para a diminuição da emissão de gases efeito de estufa através da redução de custos de armazenamento, refrigeração e transporte dos produtos até aos centros de distribuição.

### 3 METODOLOGIA

Considerando que se trata de uma investigação exploratória recorreu-se neste estudo a métodos de investigação qualitativa, com destaque para a realização de entrevistas semiestruturadas dirigidas a produtores/distribuidores de cabazes agrícolas localizados em três concelhos pertencentes à Região Viseu Dão-Lafões, nomeadamente Viseu, S. Pedro do Sul e Tondela. Considerando que por um lado a distribuição de cabazes agrícolas é ainda incipiente, pelo menos nos concelhos referidos e por outro lado a informação acerca destes produtores agrícolas é dispersa, foram realizadas 5 entrevistas entre julho e agosto de 2020, sendo que estes promotores correspondem aqueles que pela pesquisa efetuada efetuavam distribuição de cabazes agrícolas. Embora o guião da entrevista tenha vários tópicos de perguntas, neste trabalho apresentam-se apenas os resultados referentes ao tópico “COVID-19 e efeitos da pandemia na procura”. Devido à situação da pandemia COVID-19 as entrevistas foram realizadas via on-line ou por telefone. Estas entrevistas foram igualmente gravadas, transcritas e sujeitas à análise do conteúdo. Este tipo de análise é usualmente apropriado quando a pesquisa existente acerca dum fenómeno é geralmente escassa (Hsieh & Shannon, 2005).

### 4 RESULTADOS

#### 4.1 BREVE CARATERIZAÇÃO DAS INICIATIVAS

As iniciativas de distribuição de cabazes agrícolas aqui apresentadas têm associada produção agrícola. De acordo com a tabela 1, duas iniciativas de cabazes agrícolas iniciaram a respetiva produção de cabazes há dois ou menos anos, sendo que

uma dessas iniciativas (Lafobio) apenas iniciou a sua atividade de distribuição de cabazes agrícolas durante o auge da pandemia provocado pela COVID-19, particularmente entre março e abril de 2020. A Ecoseiva, seguida da Ecos do vale, são as duas iniciativas há mais tempo no mercado. Com exceção da Ecos do vale, comum a todas as iniciativas é o facto de incorporam nos seus cabazes agrícolas maioritariamente frutas e legumes. A Ecos do Vale para além de legumes e frutas, entrega ainda carne, vinho e outros produtos. Aliás a Ecos do vale começou precisamente a distribuição de cabazes agrícolas pela carne (também biológica), sendo que apenas durante o auge da pandemia provocada pela COVID 19 começou a incorporar nos seus cabazes as frutas e legumes, justamente devido á procura. Comum a todas as iniciativas é o facto de produzirem de forma biológica (em 4 iniciativas) ou de forma mais natural, ou seja, onde a utilização de produtos químicos é reduzida ao mínimo.

Tabela 1 – Caracterização das iniciativas.

<b>Nº</b>	<b>Iniciativa</b>	<b>Localização</b>	<b>Concelhos de distribuição</b>	<b>Tipo de Produção</b>	<b>Início da distribuição</b>
1	Manuela Antunes	Viseu	Viseu, Castro Daire	Biológica	2 anos
2	Ecos do Vale	S. pedro do Sul	Espinho, Porto, Tábua	Biológica	10 anos
3	Ecoseiva	Tondela	Viseu, Tondela, Coimbra, Porto	Biológica	15 anos
4	Lafobio	S. Pedro do Sul	S. Pedro do Sul	Biológica	6 meses
5	Prove – promover e Vender	Viseu	Viseu	“Natural”	8 anos

Fonte: Própria com base nas entrevistas feitas entre julho e agosto de 2020.

A Ecoseiva é a iniciativa mais abrangente em termos de locais de distribuição já que faz a distribuição em 4 concelhos (Viseu, Tondela, Coimbra, Porto), seguida da Ecos do Vale que faz a distribuição de cabazes em três concelhos (Espinho, Porto, Tábua).

Interessante ainda é observar 4 das iniciativas são individuais, ou seja gerida pelos seus promotores. Uma das iniciativas (Prove)<sup>2</sup>, embora gerida por um produtor local, integra uma rede de distribuição de cabazes agrícolas a nível nacional.

Não obstante o cabaz agrícola seja constituído por produtos agrícolas/agro-alimentares dos próprios produtores, em todas as situações há o recurso à produção oriunda de produtores locais. Esta situação é claramente visível na iniciativa PROVE,

<sup>2</sup> A rede de cabazes Prove é uma iniciativa que pretende contribuir para o escoamento da produção dos pequenos produtores familiares, fomentando as relações de proximidade entre quem produz e quem consome, estabelecendo circuitos curtos de comercialização entre pequenos produtores agrícolas e consumidores (Prove, 2020).

cujo cabaz é constituído pela produção de cinco pequenos produtores agrícolas locais, mas também no caso da Ecos do Vale, cuja produção provém largamente da Quinta da Comenda, mas também de produtores locais.

## 4.2 IMPACTOS DA PANDEMIA COVID 19 NA DISTRIBUIÇÃO DE CABAZES

À semelhança do que acontece no geral no país, onde algumas evidências referem que o estado de emergência causado pela pandemia levou a um pico na procura dos cabazes de frutas e vegetais vendidos diretamente por produtores aos consumidores (e.g., Moutinho, 2020), também a investigação em curso evidencia o aumento da procura de cabazes agrícolas durante o pico da pandemia:

*“Aumentou 100% o cabaz porta à porta (...) E muitos dos clientes já eram clientes do mercado e deixaram de ser e preferem o produto em casa” (P1)*

*“E isto cresceu muito rapidamente. Quando ia ao facebook os clientes perguntavam olhe tem legumes também, não quero pedir só carne (...) até havia mais encomendas do que aquelas que eu podia levar (...)” (P2).*

*“Houve um aumento de clientes durante o período da pandemia. Houve sim senhora. As pessoas pediram inclusivamente que a gente lhe fosse levar o cabaz a casa (P5)”.*

Se por um lado a procura de cabazes agrícolas pode diminuir com o desconfinamento social, por outro lado, pode-se esperar que esta tendência da procura de cabazes agrícolas se mantenha em termos gerais, até porque os produtos em causa são produtos com uma qualidade superior (biológicos ou mais naturais) e são também estabelecidas relações de confiança e amizade entre o produtor e o consumidor: *“E depois trocam-se ideias e o convívio é muito giro. É a família PROVE” (P5).*

Naturalmente para que esta tendência de procura de cabazes agrícolas se mantenha as estratégias de marketing utilizadas pelos promotores de cabazes agrícolas e também o relacionamento estabelecido com os clientes no ato de entrega dos produtos, assume-se como particularmente relevante. Efetivamente a pandemia resultante da COVID 19 veio apelar ao cumprimento de determinadas regras em termos de segurança e higiene, que os promotores em causa tentam cumprir escrupulosamente. Comum a todas as iniciativas é o uso de máscaras e gel para desinfetar as mãos no ato de entrega do cabaz agrícola. Um cuidado especial prende-se também com o material em que é entregue o cabaz – ou constituída por material que permite o contacto com os alimentos e é fácil lavagem ou de utilização única: (...) *para além das proteções individuais e das desinfecções nos cabazes tentamos usar materiais descartáveis, caixas de cartão que é para as pessoas depois não terem que reutilizar e não terem que devolver (P3).*

Há situações ainda onde o pagamento do cabaz é feito por transferência bancária ou o dinheiro é colocado num envelope, evitando-se assim o contacto com as mãos:

*“Na questão do dinheiro. Eu deixava o cabaz no jardim e deixavam o dinheiro num envelope ou num saco de plástico. Nos prédios acontecia o mesmo” (P1);*

*“Eles (clientes) fazem transferências depois e não há problemas para mim” (P2).*

Vale a pena ainda referir que o contacto social é evitado ao máximo como a seguir se elucida:

*Tenho uma cliente que ela vive no prédio e ela quer que ponha o cabaz no elevador (...) ela encomenda coisas todas as semanas e nunca vi esta senhora. Ela não quer contacto nenhum. Para mim está bom (P2);*

*“ (...) evitávamos o mínimo de contacto e optámos por não receber em dinheiro e tentar que as pessoas fizessem transferências ou pagassem por multibanco” (P3).*

## 5 DISCUSSÃO, CONCLUSÃO E PISTAS PARA PESQUISAS FUTURAS

Com base numa investigação exploratória, esta investigação pretende observar os efeitos que a pandemia provocada pelo COVID-19 trouxe ao nível do consumo de produtos agrícolas e agro-alimentares locais. Embora as evidências apuradas precisem ser aprofundadas, as entrevistas realizadas sugerem o crescimento da procura de produtos locais e a preferência pelas cadeias curtas de distribuição, permitindo assim um contacto entre produtor e consumidor.

De facto as práticas modernas de agricultura e a urbanização têm levado a uma complexa cadeia de distribuição, geralmente requerendo circuitos longos de transporte, envolvendo mais danos ambientais e um decréscimo da qualidade dos produtos (Bakalis et al., 2020). Os circuitos longos de distribuição, tanto em termos de distância como de tempo, envolvem ainda o manuseamento dos produtos por muitas pessoas, aumentando assim o possível risco de infeção. No entanto, a juntar ao referido, o receio e medo das pessoas em saírem de casa, nomeadamente durante o auge da pandemia fez disparar a procura das produções locais e o recurso aos circuitos curtos de proximidade.

Assim, como se evidenciou, uma potencial resposta para esta crise pode ser dada pelos circuitos curtos de proximidade e produções locais, não dependentes do comércio internacional, estando para além do mais enraizadas no território e próximas do consumidor (Cappelli & Cini, 2020).

Naturalmente, as medidas de higiene e segurança ao longo da distribuição têm que ser integralmente cumpridas. Mas é ainda importante o apoio de políticas públicas

e privadas que suportem os investimentos necessários para a difusão destes circuitos curtos de proximidade (Henry, 2020) e deste aparente regresso ao passado. Com efeito a disponibilização de incentivos fiscais e subsídios para pequenos agricultores que adotam o modelo de circuito curto, reduzindo seus custos operacionais, por parte dos governos locais deve ser tomada em consideração. Cumulativamente, a criação de programas de apoio ao escoamento de produtos locais e orgânicos, é outra medida a equacionar pelos municípios.

Mas se por parte da governança local este apoio é importante, as medidas de educação e sensibilização acerca dos produtos locais deve ser enriquecida. Por exemplo, iniciativas educacionais em escolas e comunidades para promover o consumo de produtos de proximidade e a sustentabilidade alimentar, são valiosas. Outra medida interessante a explorar, diz ainda respeito às parcerias entre restaurantes e os fornecedores de produtos agrícolas locais.

Não obstante a pandemia esteja ultrapassada, é importante considerar a vulnerabilidade e instabilidade dos mercados de abastecimento agrícola, afetados por vários fatores, o que enfatiza ainda mais as produções locais.

Naturalmente este trabalho têm limitações, das quais se salienta as relacionadas com questões temporais e o pequeno número de entrevistas realizadas aos promotores de cabazes agrícolas. Uma pista para pesquisa futura seria assim estender este estudo a outros promotores de cabazes agrícolas ao nível da região em causa ou de outras.

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# CAPÍTULO 8

## PARÂMETROS FITOTÉCNICOS DE CANA-PLANTA E DE PRIMEIRA SOCA EM SOLO ARGILOSO

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**RESUMO:** O Brasil se destaca mundialmente na produção de cana-de-açúcar. Programas de Melhoramento Genético e adoção de práticas agrícolas adequadas contribuem para alavancar a cultura no país. O presente trabalho teve por objetivo avaliar parâmetros fitotécnicos que compõem o desenvolvimento e potencial produtivo de cinco genótipos de cana-de-açúcar em ciclo de cana-

planta e de primeira soca (G1-RB0366145xMP, G2-RB996519xRB996961, G3-TUC71-7xRB036066, G4- RB036152xTUC71-7, G5-RB886952xRB986960), em solo argiloso, através da análise do diâmetro de colmo, altura do colmo principal e produtividade. O experimento foi realizado Centro Técnico de Irrigação – CTI no município de Maringá, região Noroeste do Paraná, nas safras 2018/2019 e 2019/2020. Foi possível concluir que, houve variação nos parâmetros fitotécnicos avaliados. O genótipo G2 (RB996519xRB996961) foi o mais produtivo.

**PALAVRAS-CHAVE:** Cana-de-açúcar. Desempenho. Genótipos. Produtividade.

### 1 INTRODUÇÃO

O Brasil é considerado o maior produtor de cana-de-açúcar no mundo e a cultura possui grande importância para o agronegócio nacional. A área plantada no ano de 2019 foi de aproximadamente 10,04 milhões de hectares (CONAB, 2021) e a recente demanda por etanol tem contribuído por novas áreas de expansão, além da particularidade do canavial ser renovado periodicamente.

Com a expansão da cana-de-açúcar para regiões não tradicionais de cultivo em diferentes tipos de solo e, considerando toda a variabilidade e estratificação de ambientes,

os genótipos de cana-de-açúcar deverão ser adaptadas às condições específicas de solo e clima de cada região (SANTOS, 2008).

Características de crescimento, desenvolvimento e produção da cana-de-açúcar estão diretamente relacionadas com o desempenho produtivo de cada variedade. Desta maneira, experimentos de determinação de características ligadas à produtividade de genótipos de cana-de-açúcar são realizados para avaliar o comportamento destes em diferentes safras.

O presente trabalho tem por objetivo avaliar alguns parâmetros filotécnicos de genótipos de cana-de-açúcar em ciclo de cana-planta e de primeira soca, cultivados em solo argiloso.

## 2 MATERIAIS E MÉTODOS

Foram conduzidos dois experimentos, em ciclo de cana-planta e de primeira soca, no Centro Técnico de Irrigação – CTI no município de Maringá, região Noroeste do Paraná, coordenadas geográficas latitude 23° 11' S e longitude 52° 03' W, e altitude 380 m. O clima da região é do tipo Cfa, de acordo com a classificação de Köppen. O solo da área experimental é classificado como um Latossolo Vermelho distroférico (EMBRAPA, 1999). Os experimentos foram conduzidos no delineamento experimental inteiramente ao acaso, com quatro repetições. Os tratamentos foram constituídos por cinco genótipos (G1-RB0366145xMP, G2-RB996519xRB996961, G3-TUC71-7xRB036066, G4-RB036152xTUC71-7, G5-RB886952xRB986960) de cana-de-açúcar. Cada parcela experimental foi composta por cinco linhas de cinco metros de comprimento com espaçamento na entre linha de 1,50 m, totalizando uma área total de 37,5 m<sup>2</sup>.

A coleta dos dados foi proveniente das três linhas centrais de cada parcela sendo considerado um metro de plantio para as avaliações de cada época.

Foram avaliados os seguintes parâmetros em plantas individuais: (1) Diâmetro do colmo principal, tomado por meio de paquímetro no quarto internódio; (2) Altura do colmo principal, a partir da base do colmo até o primeiro “dewlap” visível; (3) Produtividade obtida pelo número de canas por metro x massa de um colmo x fator espaçamento.

Os dados obtidos foram submetidos a análise de variância e as médias foram comparadas pelo teste de Tukey, em nível de 5% de probabilidade.

## 3 RESULTADOS E DISCUSSÃO

Houve divergência nos resultados para as variáveis analisadas nas duas safras analisadas.

Conforme demonstrado na Tabela 1, o diâmetro do colmo do genótipo G5 foi significativamente superior ao G3, na safra 2018/19.

Tabela 1: Resultados médios de parâmetros fitotécnicos de 5 genótipos de cana-planta, Maringá, Estado do Paraná, Safra 2018/19.

Genótipos	Diâmetro (mm)	Altura (m)	Produtividade (Kg ha <sup>-1</sup> )
G1 - RB0366145xMP	24,48 ab	2,33 ab	99111,5 e
G2 - RB996519xRB996961	29,70 ab	2,87 ab	189899,0 a
G3 - TUC71-7xRB036066	23,43 b	2,92 a	116043,8 c
G4 - RB036152xTUC71-7	26,73 ab	2,14 b	106030,2 d
G5 - RB886952xRB986960	31,60 a	2,40 ab	151171,0 b

Médias seguidas de mesma letra na coluna não diferem entre si pelo teste de Tukey em nível de 5% de probabilidade.

Em relação à altura do colmo principal de cana-planta (Tabela 1), o genótipo G3 apresentou superioridade (2,92 m).

Para a variável produtividade em cana-planta, safra 2018/19, o genótipo G2, seguido pelo G5 foi significativamente superior aos demais (Tabela 1). Vale destacar que, o resultado de superioridade em produtividade do genótipo G5 foi similar ao observado para o diâmetro do colmo. Segundo Silva *et al.* (2014) e Morais *et al.* (2017), o diâmetro do colmo é um dos principais componentes que se correlaciona com a produtividade dos colmos.

Para os resultados de primeira soca, safra 2019/20, não foi observada diferença significativa entre os genótipos tanto para diâmetro do colmo, como para altura de plantas (Tabela 2).

Tabela 2: Resultados médios de parâmetros fitotécnicos de 5 genótipos de cana de primeira soca, Maringá, Estado do Paraná, Safra 2019/20.

Genótipos	Diâmetro (mm)	Altura (m)	Produtividade (Kg ha <sup>-1</sup> )
G1 - RB0366145xMP	25,28 a	2,55 a	77086,3 d
G2 - RB996519xRB996961	27,13 a	2,83 a	158471,8 a
G3 - TUC71-7xRB036066	25,28 a	2,62 a	99405,5 b
G4 - RB036152xTUC71-7	24,90 a	2,64 a	98498,8 b
G5 - RB886952xRB986960	23,25 a	2,16 a	123018,8 c

Médias seguidas de mesma letra na coluna não diferem entre si pelo teste de Tukey em nível de 5% de probabilidade.

Quanto à produtividade, o genótipo G2, seguido pelos G3 e G4, se destacaram em relação aos demais. Foi observada uma tendência de diminuição da produtividade em cana- soca, todavia, o genótipo G2 (RB996519xRB996961) apresentou os melhores resultados de produtividade em ambas as safras, comprovando sua superioridade.

## 4 CONCLUSÕES

Houve variação nos parâmetros fitotécnicos avaliados.

O genótipo G2 (RB996519xRB996961) foi o mais produtivo.

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## CAPÍTULO 9

### SEVERITY OF 'WOOD POCKET' PHYSIOPATHY IN SELECTED PERSIAN LIME PLANTS OF DIFFERENT GENERATIONS

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**ABSTRACT:** As an export product, Persian lime faces various challenges that threaten its sustainability, especially phytosanitary issues caused by fungal, bacterial, and viral pathogens that affect citrus crops in general. In addition, Persian lemon is particularly impacted by a genetic condition known as “wood pocket” which significantly weakens the tree, often leading to its death. The selection of specific plant lines helps mitigate the progression of this condition. This study aimed to evaluate the evolution of “wood pocket” across three generations of inherited and selected plants. Evaluations were conducted in three Persian lime orchards located in Michoacán, Mexico. The selection criteria were based on observations from a progenitor orchard, Selection 1 orchard, and Selection 2 orchard. In each orchard, 80 trees were randomly selected and monitored using a four-level symptomatology scale. Collected data were transformed into percentage values using the arcsine square root transformation and processed through an analysis of variance. The results revealed significant differences between the three orchards. The progenitor orchard showed the highest incidence (37.5%) at severity level 2. In Selection 1 orchard, level 1 dominated with 36.56%. In Selection 2 orchard, 91.56% of the plants exhibited minimal or imperceptible symptoms (level 1). Across generations, the incidence of levels 2, 3, and 4 decreased, while level 1

increased, indicating the potential to reduce the impact of “wood pocket” physiopathy through plant selection.

**KEYWORDS:** *Citrus latifolia*. Persian lime. Tahiti lime. Sectorial spot.

## SEVERIDAD DE LA FISIOPATÍA ‘WOOD POCKET’ EN PLANTAS SELECCIONADAS DE LIMÓN PERSA DE DIFERENTES GENERACIONES

**RESUMEN:** Como producto de exportación y ante el riesgo de impactar negativamente, el limón persa enfrenta diversos problemas que constantemente presionan su permanencia, destacan los fitosanitarios migrantes de origen patógenos, cuyos agentes fúngicos, bacteriales y virosos afectan en lo general a los cítricos. Sin embargo, en limón persa, además, una condición genética llamada “wood pocket” lo afecta gravemente. Esta condición debilita rápidamente al árbol, hasta su muerte, por lo que la restitución de plantas seleccionadas mitiga su progreso. El objetivo fue evaluar la evolución de “wood pocket” en plantas heredadas y seleccionadas en tres ciclos. La evaluación se llevó a cabo en tres huertas limón persa de la zona productora de Michoacán, México. El criterio de selección, derivó de una huerta progenitora, una huerta de selección 1 y una huerta de selección 2. En cada huerta se monitorearon 80 árboles por cada línea de cuatro al azar. En ellos, se buscó sintomatología basada en una escala diseñada de cuatro niveles. Los datos obtenidos fueron porcentuales y transformados al arcoseno de la raíz cuadrada de la proporción, y procesados por análisis de varianza. El análisis detectó diferencias para las tres huertas observadas. Según características sintomáticas, en la huerta progenitora, el nivel 2 tuvo mayor incidencia (37.5%) de plantas afectadas. En la huerta selección 2, el nivel 1 sobresalió con 36.56%. En la huerta selección 3, el nivel 1 se obtuvo en el 91.56% de plantas con síntomas imperceptibles. Al comparar el resultado entre huertas, los niveles 2, 3 y 4 tendieron a disminuir, en cambio el nivel 1 tendió a incrementar. Así, es posible acotar la incidencia de plantas afectadas por la fisiopatía de “wood pocket” a través de selección de plantas.

**PALABRAS CLAVE:** *Citrus latifolia*. Lima persa. Limón Tahití. Mancha sectorial.

### 1 INTRODUCTION

According to the FAO, in 2021, Mexico ranked second worldwide in harvested citrus area, with 195,619 hectares, only behind India, which reported 327,000 hectares. In terms of production, Mexico yielded 2,983,802 tons, compared to 3,548,000 tons in India. However, regarding yield per hectare, Mexico ranked 39th, with an average of 15.25 tons per hectare (FAOSTAT, 2023). Mexican lime represents 70% of the national citrus production, while Persian lime accounts for the remaining 30%, meeting both national and international market demands. Mexico is one of the world’s leading citrus producers and exporters, with the United States as the primary importer, followed by Japan (Ruiz *et al.*, 2017). In 2022, the most productive states were Veracruz (27.7%), Michoacán (27.6%), and Colima (10%) (SIAP-SADER, 2023).

Persian lime (*Citrus latifolia*), a round, seedless hybrid fruit, is larger and sweeter than a typical lime. Its characteristic acidic juice makes it ideal for beverages, nectars, and food preparation. It is also a rich source of vitamin C, providing 64% of the daily recommended value in a 100-gram serving. Additionally, Persian lime contains numerous phytochemicals, such as polyphenols, terpenes, and tannins, and has significant concentrations of citric acid. Its composition is 88% water, 10% carbohydrates, and less than 1% fats and proteins.

Due to its economic significance (Almaguer-Vargas *et al.*, 2011), Persian lime is constantly under pressure from various phytosanitary challenges, including pathogens of fungal, bacterial, and viral origin (Hernández-Mora *et al.*, 2023). Moreover, a genetically-based abiotic disease known as “sectorial spot” or more commonly, “wood pocket” severely affects this citrus variety, particularly in warm climates. In the field, the symptoms of “wood pocket” are often mistaken for those of Huanglongbing (HLB) disease in citrus (Villegas and Mora, 2011). The interval between the onset of symptoms and tree death can be as short as one month, though it often takes longer. Some trees exhibit symptoms while others remain symptom-free, and it is possible to find both healthy and affected fruits on the same plant. The symptoms include leaf mottling, branch cracks, and spots on fruits, all of which weaken the tree until it dies.

Although most known Persian lime clones are susceptible to “wood pocket” the physiopathy manifests only in hot, dry regions. In other climates, the plant behaves normally. Selecting specific plants helps mitigate the progression of the physiopathy. Therefore, this study aimed to evaluate the evolution of “wood pocket” in inherited and selected plants over three cycles.

## 2 MATERIALS AND METHODS

The evaluation was conducted in Persian lime orchards belonging to a cooperating farmer in Felipe Carrillo Puerto, also known as “La Ruana,” in the municipality of Buenavista, Michoacán, Mexico. The area is located at an average altitude of 400 meters above sea level. The region’s climate is classified as Bs<sub>1</sub>, corresponding to the least dry of the dry climates (García, 2004). Vegetation consists of deciduous tropical forest species, and the soil type is pellic Vertisol (INEGI, 2016).

Three orchards were selected to explore the presence of “wood pocket” symptoms. The first orchard, known as the progenitor, was approximately nine years old. From this orchard, the second orchard was derived, referred to as Selection 1 (first generation), which was about six years old. Similarly, the third orchard, known as Selection 2 (second generation), was derived from the previous one.



Visual monitoring was carried out to identify the characteristic symptoms of “wood pocket” in each orchard. A four-level severity scale (Table 1) was designed to assess the observable symptoms in the sampled Persian lime plants.

Table 1. Scale used to evaluate the level of impact (severity) of symptoms related to “wood pocket” physiopathy in Persian lime.

Level	Extended visual characteristics	Short description
1	Imperceptible symptoms	No visible symptoms
2	Leaf wrinkling on branches and/or noticeably dry branches	Some dry branches
3	Dry branches, sectorial leaf mottling, and vertical spots on fruits	Strong symptoms
4	Significant leaf loss, exposed or dry branches, and tree decline	Severely affected

In each orchard, visual exploration was performed on 80 productive plants across four random lines (320 plants per orchard). The severity of “wood pocket” was measured using the four-class scale. The data were transformed using the arcsine square root of the proportion and analyzed through a randomized block design and factorial analysis. The statistical software used was SAS 9.2 (2019).

### 3 RESULTS AND DISCUSSION

Table 2 presents the mean values obtained from the variance analysis. Since the data were transformed to percentages to detect accurate differences in symptom severity levels, the results reflect these transformations. In the progenitor orchard, severity level 2, which corresponds to “some dry branches,” was the most prevalent, affecting a significant number of plants. Meanwhile, in Selection 1 orchard, severity level 1, meaning “no visible symptoms,” was observed most frequently. However, statistically, severity levels 1, 2, and 3 were not significantly different from each other. In Selection 2 orchard, severity level 1 was clearly the most common, indicating a reduction in symptom progression across generations (Table 2).

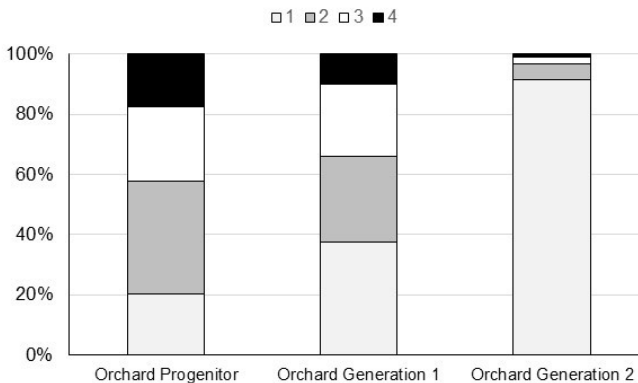
Additionally, non-transformed severity data are shown in Figure 1, highlighting the large proportion of plants in Selection 2 orchard with level 1 symptoms (“no visible symptoms”). This suggests a noticeable decrease in the occurrence of “wood pocket” over time.

Table 2. Variance analysis conducted in progressive orchards for the presence of “wood pocket” physiopathy in Persian lime.

Symptomatic level	Orchard <sup>†</sup>		
	Progenitor <sup>*</sup>	Selection 1	Selection 2
1	0.46 b	0.64 a	1.27 a
2	0.65 a	0.55 ab	0.23 b
3	0.51 b	0.50 b	0.14 bc
4	0.43 b	0.31 c	0.06 c
C.V.	8.30	12.79	12.42
<i>P</i>	0.0002	0.0003	0.0001

<sup>†</sup>Data previously transformed to arcsine of the square root of the ratio; <sup>\*</sup>Means followed by the same letter within columns do not differ statistically (Tukey, 0.05).

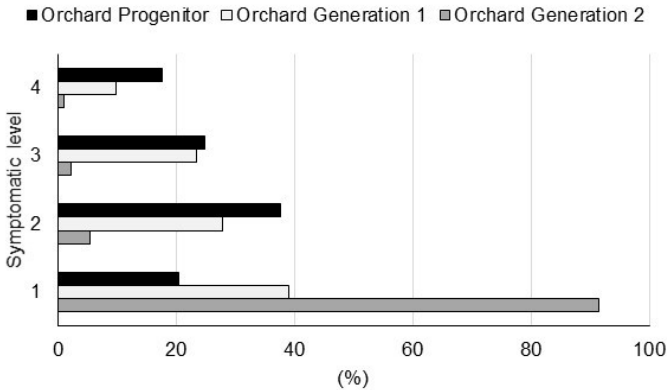
Figure 1. Percentage distribution derived from the statistical analysis carried out in the orchards for the presence of “wood pocket” physiopathy in Persian lime.



The comparison of the three generations (Figure 2) shows that the progenitor orchard reached its highest symptomatic incidence (37.5%) at severity level 2. In contrast, severity level 4 (most severe) affected only 17.5% of the plants in this orchard. In Selection 1 orchard (first generation), severity level 1 was the most frequent (36.56%), while severity level 4 affected only 9.68% of plants. In Selection 2 orchard (second generation), 91.56% of plants showed no visible symptoms (severity level 1), while only 0.93% of plants were categorized as severity level 4.

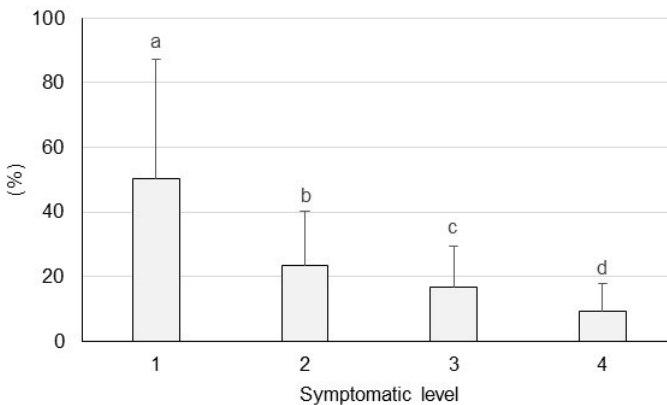
In general, severity levels 2, 3, and 4 tended to decrease across generations, while severity level 1 increased (Figure 2). This trend suggests that selecting specific plants can effectively reduce the impact of “wood pocket.”

Figure 2. Presence of “wood pocket” physiopathy in Persian lime: comparative range analysis of the three orchards.



Further analysis was conducted to explore the interaction between orchard (H) and symptom level (N) as independent factors. Significant statistical differences were detected for the H × N interaction. When analyzed independently, the orchard factor (H) did not show significant differences, but the symptom level factor (N) did, as shown in Figure 3. This analysis confirmed that severity level 1 represented the highest proportion of plants, followed by the other severity levels. Thus, although “wood pocket” intensity decreases, it remains present in varying degrees across orchards.

Figure 3. Separate analysis of variance for the symptomatic level (N) factor of “wood pocket” physiopathy in Persian lime.



On the other hand, the analysis of variance performed on the combined treatments (H/N) for the incidence of “wood pocket” revealed statistical differences. The highest number of healthy plants was observed in the H3/N1 combination. In the remaining combinations, “wood pocket” was still present, though its incidence tended to decrease with each generation (Table 3).

Table 3. Combined analysis of variance for the effect of orchard (H) and symptomatic level (N) factors on “wood pocket” physiopathology in Persian lime.

Progenitor Orchard †		Selection 1 Orchard		Selection 2 Orchard	
H1/N1	0.47 ed	H2/N1	0.67 b	H3/N1	1.27 a
H1/N2	0.66 cb	H2/N2	0.55 cd	H3/N2	0.23 gf
H1/N3	0.52 ed	H2/N3	0.50 ed	H3/N3	0.14 gh
H1/N4	0.43 e	H2/N4	0.31 f	H3/N4	0.68 h

†Data were previously transformed using the arcsine square root of the proportion; means followed by the same letter within columns do not differ statistically (Tukey, 0.05); C.V. 9.51; Significance \*\*.

The economic potential of Persian lime has been prematurely overshadowed by the emergence of this phytopathy, which defoliates plants, dries branches, stains fruits, and ultimately leads to the death of affected trees. It is crucial to identify the early symptoms to mitigate the effects of stress and other factors that trigger the manifestation of “wood pocket” (Ríos-Rojas *et al.*, 2018).

Although the specific physiology associated with the response of plants exposed to high temperatures and the incidence and severity levels of “wood pocket” remains unknown (Rodríguez *et al.*, 2020), an unidentified genetic component causes varying reactions among plants of the same clone. In an affected plantation, severely impacted trees may coexist alongside others that appear healthy. The time between the onset of symptoms and plant death has not yet been clearly established; it can range from a few weeks to several months, and not all individuals in a population exhibit the same symptoms.

## 4 CONCLUSIONS

The severity levels of “wood pocket” symptoms varied between orchards, gradually decreasing over generations. As the incidence of affected plants declined, the proportion of plants at severity level 1 increased to 81%, 77%, and 53% for levels 4, 3, and 2, respectively. This indicates that selecting specific plant lines helps control the impact of “wood pocket.”

## 5 ACKNOWLEDGMENTS

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# CAPÍTULO 10

## THE DILEMMA OF THE DEVELOPMENT OF OIL PALM PLANTATIONS AGAINST FOREST CONSERVATION IN CAMEROON

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**ABSTRACT:** The economic crisis of the 1980s coupled with the devaluation of the CFA franc in 1994 led to significant impoverishment of rural populations with a significant drop in purchasing power. Such a phenomenon has driven to the emergence of new income-generating activities. This is how the rural and elite populations of some administrative divisions or subdivisions (Ngwéi, Ekondo Titi) rushed to the production of palm oil since 1990, both for food needs and for cash income. The cultivation of oil palm leads to the clearing of large areas of dense forest each year in Cameroon. As such, oil palm cultivation becomes the main driver of deforestation leading to the loss of plant and animal biodiversity as well as soil and water pollution, which raises questions about its sustainability. The oil palm sector is progressing in Cameroon with around 360,000 ha of palm plantation shared between agro-industries, elites and small farmers. Annual palm oil production increased from 270,000 tons in 2013 to 413,000 tons in 2018 against a demand that peaked at 1,179 million tons in 2018. As methodology is concerned, we use geospatial tools through multi-temporal and multi sensor satellite images like Landsat from 1976 to 2015, IKONOS, GEOEYE, Google Earth from 2009 to 2016 to map the dynamic of different forms of land use and land cover within those areas. Secondly field investigations allow us to collect field data on the number producers, the areas per producer, the production itself

as well as the validation of the results of images processing. The results show that oil palm cultivation has both positive and negative impacts, however the negative impacts are numerous and difficult to correct, hence the dilemma. Indeed, in terms of positive impacts we can mention employment and income then the improvement of the living conditions of certain households and elites in particular; while in terms of negative impacts, we will readily cite deforestation, the intensive use of fertilizers and pesticides which lead to the pollution of waterways, and the difficult disposal of waste from presses. Also a double analysis by the impacts and by political ecology makes it possible to better analyze the causes of such a phenomenon while highlighting the essential consequences of the management and transformation of the environment. Also, with more than 820ha of forest lost per year, oil palm become the main and pernicious driver of deforestation. The negative effects degradation obviously relate to the fragmentation of ecosystems and habitats; pollution or contamination of river water used for food and body needs in the absence of drilling, impoverishment of soils and their erosion (sloping soils), conversion of degraded lands and reduction of carbon stocks through the attack on marshy and malaria areas, the social divisions and injustices born from the purchases and dispossession of land by the elites and the recruitment of foreign labor, the loss of biodiversity and food security (lack of time and land) of rural populations, return migrations and local migrations towards areas of high production. However, it is necessary to maximize the positive impacts and minimize the negative impacts and here too the dilemma arises, because what type of governance can lead to a win-win oil production system. Should we not identify degraded lands and allocate them to oil palm then identify those still intact for protection; tourism and municipal forestry? Furthermore, how can we remain indifferent to the conversion of forests of very high conservation value where much sought-after primates nest? This paper aim at analyzing the dilemma and the controversy of elaeisfarming following the massive destruction of dense forest and high conservation value forest in Cameroun. The most suitable areas of elaeisfarming is main center of endemism in Cameroon (another dilemma) and as such, it threat destruction of biodiversity while compromising conservation. It raises up a problem of governance which implies a better articulation of the tensions between development and environmental issues.

**KEYWORDS:** Conservation. Deforestation. Dilemma. Elaeisfarming. Impacts.

## O DILEMA DO DESENVOLVIMENTO DAS PLANTAÇÕES DE PALMA DE ÓLEO VERSUS A CONSERVAÇÃO FLORESTAL NOS CAMARÕES

**RESUMO:** A crise económica dos anos 80, juntamente com a desvalorização do franco CFA em 1994, conduziu a um empobrecimento significativo das populações rurais, com uma queda significativa do poder de compra. Este fenómeno levou ao aparecimento de novas actividades geradoras de rendimento. Isto é como as populações rurais e de elite de algumas divisões ou subdivisões administrativas (Ngwéi, Ekondo Titi) correram para a produção de óleo de palma desde 1990, tanto para necessidades alimentares como para rendimentos monetários. O cultivo do dendezeiro leva à desflorestação de grandes áreas de floresta densa todos os anos nos Camarões. Como tal, o cultivo de palma torna-se o principal motor da desflorestação, levando à perda de biodiversidade vegetal e animal, bem como à poluição do solo e da água, o que levanta questões sobre

a sua sustentabilidade. O sector do óleo de palma está a progredir nos Camarões, com cerca de 360.000 hectares de plantação de palma partilhados entre agro-indústrias, elites e pequenos agricultores. A produção anual de óleo de palma aumentou de 270 mil toneladas em 2013 para 413 mil toneladas em 2018, contra uma procura que atingiu o pico de 1,179 milhões de toneladas em 2018. No que diz respeito à metodologia, utilizámos ferramentas geoespaciais através de imagens de satélite multitemporais e multisensoriais como o Landsat de 1976 a 2015, IKONOS, GEOEYE, Google Earth de 2009 a 2016 para mapear a dinâmica das diferentes formas de uso e cobertura do solo dentro destes áreas. Em segundo lugar, as investigações de campo permitem-nos recolher dados de campo sobre o número de produtores, as áreas por produtor, a produção em si, bem como a validação dos resultados do processamento de imagem. Os resultados mostram que o cultivo do dendezeiro tem impactos positivos e negativos, no entanto os impactos negativos são numerosos e difíceis de corrigir, daí o dilema. Com efeito, em termos de impactos positivos podemos referir o emprego e o rendimento, depois a melhoria das condições de vida de certos agregados familiares e das elites em particular; enquanto que em termos de impactos negativos citaremos prontamente a deflorestação, o uso intensivo de fertilizantes e pesticidas que levam à poluição dos cursos de água e a difícil eliminação dos resíduos das prensas. Também uma dupla análise pelos impactos e pela ecologia política permite analisar melhor as causas de tal fenómeno, ao mesmo tempo que destaca as consequências essenciais da gestão e transformação do ambiente. Além disso, com mais de 820 hectares de floresta perdidos por ano, o dendezeiro tornou-se o principal e pernicioso motor da deflorestação. Os efeitos negativos da degradação estão obviamente relacionados com a fragmentação dos ecossistemas e dos habitats; poluição ou contaminação das águas dos rios utilizadas para alimentação e necessidades corporais na ausência de perfuração, empobrecimento dos solos e sua erosão (solos inclinados), conversão de terras degradadas e redução dos stocks de carbono através do ataque a zonas pantanosas e de malária, as divisões sociais e as injustiças nascidas das compras e expropriação de terras pelas elites e do recrutamento de mão-de-obra estrangeira, da perda de biodiversidade e de segurança alimentar (falta de tempo e de terras) das populações rurais, das migrações de retorno e das migrações locais para áreas de elevada produção. Contudo, é necessário maximizar os impactos positivos e minimizar os impactos negativos e também aqui surge o dilema, porque que tipo de governança pode levar a um sistema de produção petrolífera ganha-ganha. Não devemos identificar as terras degradadas e alocá-las ao dendezeiro e depois identificar aquelas que ainda estão intactas para proteção; turismo e silvicultura municipal? Além disso, como podemos permanecer indiferentes à conversão de florestas de altíssimo valor de conservação, onde nidificam primatas muito procurados? Este artigo tem como objetivo analisar o dilema e a controvérsia da elaeisfarming após a destruição maciça de florestas densas e de elevado valor de conservação nos Camarões. As áreas mais adequadas para a agricultura elaeis são o principal centro de endemismo nos Camarões (outro dilema) e, como tal, ameaçam a destruição da biodiversidade, ao mesmo tempo que comprometem a conservação. Levanta um problema de governança que implica uma melhor articulação das tensões entre o desenvolvimento e as questões ambientais.

**PALAVRAS-CHAVE:** Conservação. Deflorestação. Dilema. Elaeisfarming. Impactos.



## 1 INTRODUCTION

### 1.1 CONTEXT AND PROBLEM

Agriculture is one of the main causes of the degradation of natural ecosystems (Bahuchet. & Betsch 2012). It accounts for 24% of global greenhouse gas emissions Carlson et al; (2013). The resulting climate changes affect the whole humanity IPCC (2007). Agriculture is also the primary anthropogenic cause of deforestation and desertification. It largely participates in the degradation of water resources with the increased use of chemical inputs (Tchindjang et al, 2016; Tchindjang, 2017). These negative impacts are mainly imputable to industrial agriculture, practiced over large areas and without taking into account the basic principles of sustainability. Artisanal agriculture also presents unsustainable practices such as shifting slash-and-burn agriculture (Bahuchet. & Betsch 2012; Zhang et al, 2002).

On a global scale, palm oil is the leading vegetable oil for consumption since 2015, and its demand continues to grow. The processed food industry consumes approximately 72% of all palm oil production, the personal care and cleaning products industry consumes 18%, and the biofuel industry consumes the balance at 10%. The largest exporting countries of crude palm oil and its fractions (whether or not refined) in 2017 were Indonesia (USD 18.7 billion), Malaysia (USD 9.8 billion), and the Netherlands (re-export) (USD 1.2 billion), while the largest importing countries that year were India (USD 6.5 billion), China (USD 3 billion), and Pakistan (USD 2.2 billion) Brack et al. 2016, Workman, 2019a&b). The multiplication of uses of palm oil in cosmetics, energy (biofuels), agri-food, etc. amplifies global and national demand.

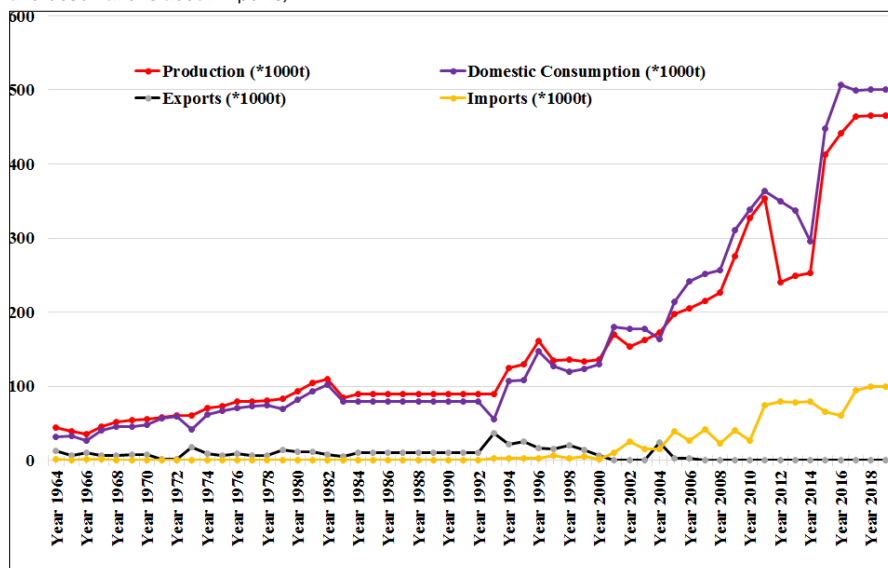
In Africa, Cernansky (2019) thought that an estimated 3.03 million ha of land “traditionally used or inhabited by local communities,” covering both forest and farmland, have been acquired by palm oil companies. Cameroon is a middle income country with about 70% to 75% of its population dependent on the agricultural sector for livelihood and employment. Agricultural sector provides about 30% of the country’s GDP (Bassel, 2014; Bassel and Sisak, 2014) Cameroon is a cradle for palm oil production and being amongst the main producers of palm oil in Africa has attracted much attention recently from private companies worldwide.

Palm oil is not new to Cameroon with the first commercial plantations being established in 1907 under the German colonial administration in the coastal plains, around Mt. Cameroon and Edéa (Ndjogui et al, 2014, Ngom et al, 2014). The crop was further developed under the Franco-British regime until 1960 when it had reached an estimated production of 42,500 tons (Carrere, 2013). After Independence, the government of

Cameroon took over palm oil production with the creation of public sector companies like “SOCAPALM, PAMOL and CDC. The Cameroonian production level positioned the country as the 13th producer of palm oil in the world and the leading producer in the Central African sub region (USDA, 2020). This considerable increase palm oil production was mainly due to the dropping prices of cocoa and coffee which were then the major cash crops. The economic crisis in the late 1980s and early 1990s, including the devaluation of the CFA franc impacted many smallholders in the ecologically suitable areas driving a shift to the development of oil palm plantations (Ngando et al, 2011).

Cameroon’s palm oil production is growing fast since 1993 and was estimated to 465,000 tons of palm oil in 2019 (USDA, 2020) while consumption and import grew to reach respectively 500,000 tons and 100, 000 tons (Figure 1).

Figure 1: Palm oil production, consumption and export/import in Cameroon (Source, USDA, 2020 complete by field work and observations about imports).



Cameroon ranks 10th among palm oil producing countries in the world and 3rd in Africa behind Nigeria (940,000 tons) and Ivory Coast (417,000 tons). Its production is estimated reach more than 650 000 tons in 2022 for a national demand estimated at more than 1,179, 000 tons (Miaro et al, 2020). The sector functions with a structural deficit of 160, 000 tons.

Unfortunately, the increase in cultivated areas comes at the expense of natural forest ecosystems with high conservation value and plots allocated to other activities such as agriculture. Elaeis farming raises a lot of controversy. It induces unprecedented degradation and deforestation. The losses of biodiversity are inestimable in a context

where concerns on a global scale are oriented towards the conservation and preservation of ecosystems useful for mitigating climate change, and therefore, for the sustainability of life on earth. Also, it contributes to the decline or at least the stagnation of other economic activities in prone cultivated areas i.e. Ngwéí, Ekondodo Titi, Sanaga Maritime. With the scarcity of land, the decline in food production and the emergence of social conflicts (particularly linked to land). Soil degradation, pollution and contamination of river water, etc. are so many other problems that the cultivation and processing of this plant generate. The dilemma is therefore obvious and lies between the socio-economic benefits and the environmental problems generated by the development of palm oil production. The ultimate concern is how to reconcile elaeiculture with the imperative need for preservation and sustainable management of ecosystems in this department. This dilemma leads the oil palm to tackle the conservation by eating away at the areas of the protected areas of the territories concerned or by downgrading them in favor of granting industrial plantations.

In Cameroon, the oil palm production is stratified in three sectors: an agro-industrial sector, smallholder's scheme and medium holder's scheme (semi-industrial) in contract with agro-industries and independent small and medium scale producers (Bakoume et al 2002). We estimates today that more than 600,000ha are used for palm oil farming in Cameroun in five types of plantations.

- Family planters (type1) with an area ranging from 1 to 15 ha.
- Rural investors (type 2) with an area around 15 to 50 ha.
- Urban investors (type 3) that are still called elites, with an area ranging from 50 to more than 200 ha.
- Private investors (type 4) from 800-5, 000ha.
- Agro industrial (type 5) plantations with more than 10,000 ha.

But this view seems too simplistic and we need to organize the production around (table 1)

Table 1: Organization of oil palm production in Cameroon.

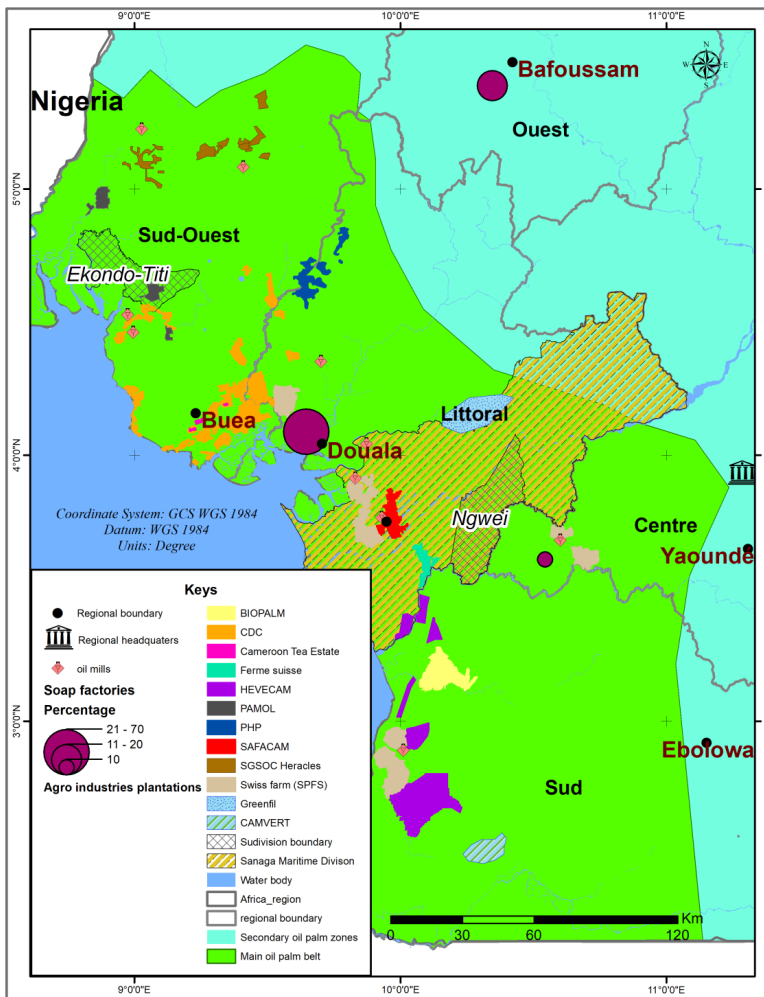
Scale	Size of the farms ha	Type of mills	Destination of the products
Smallholders & families	1-5h	Hand and foot	Family consumption and sales
Smallholders	5-15	Hands	Local consumption and sold
Motorized Smallholders	15-50	Mechanical, motorized	Wholesale, to small scale retailer /individuals
Independent holdings	50-250ha	Motorized, semi industrial	Great retailer, second transformation
Private investors	More 800-5000ha	Industrial	second transformation industries
Agro industries	More than 10000ha	Industrial	second transformation industries

## 1.2 BACKGROUND AND STUDY FRAMEWORK

This study focuses on Cameroon, a country in Central Africa located between 2°-13°N and 8°-16°E (Figure 2). This “Africa in miniature” had an estimated population of 27 million in 2020. It is a predominantly young population; the 0-15 age group representing nearly 43% of the total workforce (BUCREP, 2010). Physically, Cameroon has a dry tropical climate in the north and a humid equatorial climate in the south. The relief is very diverse, ranging from landscapes of coastal plains to high plateaus and mountain ranges scattered across the territory, not to mention a long opening (400 km) to the Atlantic Ocean. In terms of water resources, Cameroon is the second wettest country in Africa after the Democratic Republic of Congo. Economically, Cameroon is one of the middle-income countries with a human development index (HDI) of 0.563 in 2018. Economic activity is dominated by the primary sector (45% of GDP) (MINEPAT, 2009). Agriculture employs 45.3% of the working population. The diversity of the conditions of the biophysical environment is favorable to the development of a large number of crops, both food (cassava, maize, millet, macabo, rice, etc.), and cash (sugar cane, cotton, palm oil, rubber, cocoa, etc.). Regarding the oil palm, it develops preferentially in the coastal area qualified as the “elaeisfarming” belt of Cameroon (figure 2). Administratively, oil palm plantations and concessions are set up in the maritime facades of the southern, coastal and southwestern regions.

As drawn in the map, this belt offers suitable conditions for the development of oil palm: low altitude (less than 500 m); sufficient rainfall (more than 1800 mm / year); favorable temperature between 22 and 30°C; low thermal amplitude; rich and deep soils; etc. It is in this area that one can find agro-industries, the major producers of palm oil. At the edge of this “elaeisfarming” belt, there are a few small marginal farms both on the vast southern Cameroonian plateau and in the Western Highlands where oil palm could be sown with lukewarm success.

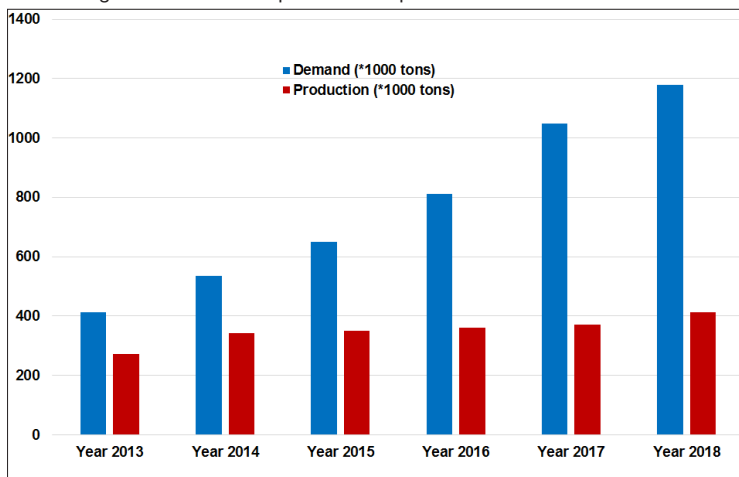
Figure 2: "elaeisfarming" areas, oil palm farms and industries of Cameroon.



Cameroon's oil palm production has grown at a rate of 2.8% yearly that is from 100,000 tons of CPO in 1990 to about 270,000 tons in 2014 (www. indexmundus .com), 372,000 tons in 2017 (Mbodiam, 2019) and 413,000tons in 2018 (SA, 2019). Despite this increase production and the efforts put in place by the government through different programs, projects and partnership with NGOs, the problem of palm oil demand (figure 3) and its related products has not been solved within the country. Palm oil is regularly imported to fill the gap, with the country registering import of about 80,000 tons in 2012, 78,000 tons in 2013 and 80,000 tons in 2014 (USDA 2020) 95000 tons in 2017, 100,000 tons in 2018. This deficit in the supply of palm oil is mainly due to aging plantations mostly made up of the wild variety (*dura*), poor management of the plantations and poor mills performance (artisanal mills). The transformation of palm fruits is done by two sectors

being industrial or artisanal, but their mills are very old with a reduced efficiency as equipment is outdated. The increasing oil demand has gone from 100,000 tons in 2010 to reach more than 1,000,000 tons<sup>1</sup> nowadays due to colossal investments and demand from second transformation industries like SCR Maya, SODECOTON, Refinery PACIFIC, AZUR, SAAGRYS, SOC, etc.

Figure 3: Demand and production of palm oil in Cameroon since 2013.



## 2 METHODOLOGY

The study began with documentary research in various libraries of institutions in Yaoundé to gain an overall understanding of the oil palm sector in Cameroon and particularly in the production basin. The emphasis is placed on the history of the sector in Cameroon and the factors and reasons for the development of this speculation in Cameroon. The second phase was developed around field surveys using questionnaires. To this end, 334 questionnaires were administered over a week to village and industrial oil producers in the eleven (11) districts of Sanaga Maritime. Further 290 and 260 questionnaires were administered in Ngwéi and Ekondo-Titi Sub-divisions respectively. These surveys placed particular emphasis on, among other things: production mechanisms, the ecological and socio-economic impacts of palm groves and the prospects for the sector in the department. Field observations, interviews and focus groups carried out with populations, local authorities and industrialists completed the approach.

The surveys carried out among palm plantation owners focused on the characteristics of the palm groves: size, varieties planted, age, production per hectare,

<sup>1</sup> EcoMatin, <https://ecomatin.net/huile-de-palme-le-deficit-de-production-du-cameroun-se-creuse-a-700-000-tonnes/> accessed on 03/08/2020

varieties cultivated, yields, evacuation of production etc., perception by the local populations of the ecological and socio-economic impacts or mutations of elaeiculture, the future of this speculation in their department... GPS surveys of palm plantations and field photographs of different issues were also carried out during the surveys.

Geopastical tools were mobilized. The processing and analysis of satellite images of Sanaga Maritime were carried out diachronically and based on Landsat, GEOEYE, IKONOS and Spot images.

Using ArcGIS and Envi and Erdas remote sensing software, the various maps were generated. The processing of multi-date images, subsequent analyzes and interpretations made it possible to understand the dynamics of land use in a relevant manner. Particular emphasis will have been placed on the evolution of the forest cover in the department in relation to the dynamics of development of elaeiculture. These analyzes and interpretations of satellite images and the resulting maps provided a global overview of the changes induced over time by the development of olive growing, housing, and other agricultural activities.

The processing of the survey questionnaires was carried out using Microsoft Excel 2007 and made it possible to generate various diagrams, providing information on numerous aspects linked to the evolution of oil palm cultivation, such as: different categories of planters, the distribution by gender of oil growers, the method of land acquisition, the origin of financing for the creation of a palm grove, the evolution of yields and income, the destination of the production, the type of vegetation welcoming palm plantations, etc.

This study, carried out between 2013 and March 2020 by an interdisciplinary team (geographers, botanists, environmentalists and geomatician specialists), highlighted the impact of oil palm cultivation on the landscape and the range of tools used during this study are resumed in Table 2.

Table 2: Methods used in assessing the impact of oil palm plantation in the environment.

<b>Methods</b>	<b>Operational work</b>
Satellite image processing	LANDSAT image processing (MSS, TM, ETM+ & 8 de 1975 à 2015)
Botanical Survey on quadra and transects	Two quadras and two transects in each type of palm plantation visited on field (village, elitist et industrial); a quadra in the dense forest.
Environmental impacts assessment	Interaction matrix and impact sheet per receiving environment
Questionnaire Survey	290 and 260 questionnaires respectively in Ngwéi and Ekondo-Titi subdivisions

Landscape method	Application of the SEPL exercises in 4 villages (two per subdivision) which involves a total of 60 participants.
Carbon stock estimation method	Assessment of carbon stock in aboveground biomass by allometric equation

In this paper, we did not take into account the carbon stock assessment.

As impact assessment is concerned, the combination of characterization criteria such as the nature, probability of occurrence, scope or extent, magnitude or intensity, reversibility and duration of the identified impacts is necessary in the classification of these impacts. The assessment of the identified impacts was done by rating (Tchindjang, 2017). Ratings from 1 to 5 were assigned to the indicators (table 3) depending on the degree of impact. The absolute importance represents the average of the impact ratings over the total number of rated indicators. Nature is the way of being of an impact which can be positive (○) or negative (●) in the concerned environment.

Table 3: Impact assessment indicators and rating de l'évaluation des impacts.

Value	Occurrence of the impacts	Territorial scope (extent) of the impacts	Duration of the impacts	Intensity of the impacts	Reversibility of the impacts	Final rafting
1	Very unlikely	Very reduce space (10%)	Very short	Very weak	Immediately reversible	1-2 non significant or negligible
2	Unlikely	Reduce space 15-20%	Temporary	Low	Quickly reversible	
3	Likely	Fairly extensive 25-40%	Long enough	Way	Reversible	2.1-2.9 insignificant
4	Certain	Extended 50%	Long	High	Little reversible	3-4 significant
5	Very certain	Very extensive 60-100%	Very long	Very high	Irreversible	4.1-5 very significant

The absolute importance or significance of the impacts is determined by calculation by taking the product of all the ratings assigned to each indicator over the total number of indicators. This is illustrated by the following equation:

$$\text{Absolute importance} = \frac{\sum \text{Ratings}(\text{intensity} \times \text{reversibility} \times \text{extent} \times \text{duration} \times \text{occurrence})}{5}$$

Equation 1

After rating the impacts were qualified according to the results obtained.

- The rating [1- 2] represents the insignificant or negligible impacts;
- The rating [2.1 - 2.9] represents the insignificant impacts;
- The rating [3- 4] represents the significant impacts;
- The rating [4.1 - 5] represents very significant impacts.



The impact criticality threshold is established as the rating value greater than or equal to the average of the grid: 3.

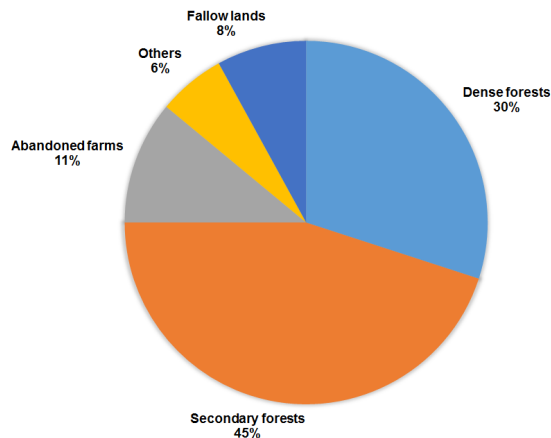
### 3 RESULTS AND INTERPRETATION

Paragraphs below allow us to address the main axes of sustainability into the oil palm sector: economic viability, social inclusion, environmental preservation and governance. We observe on field that Most of the components of the biophysical environment are affected by this activity. Globally, we notice the clearing of the forest for the establishment of new plots, the erosion of the land during exploitation, the pollution of air and water during processing as well as relative poverty and inequality among peasants. We can include grievances related to the distribution of benefits and the development of neighboring communities.

#### 3.1 OIL PALM BETWEEN DEFORESTATION AND ECOLOGICAL SUSTAINABILITY IN CAMEROON

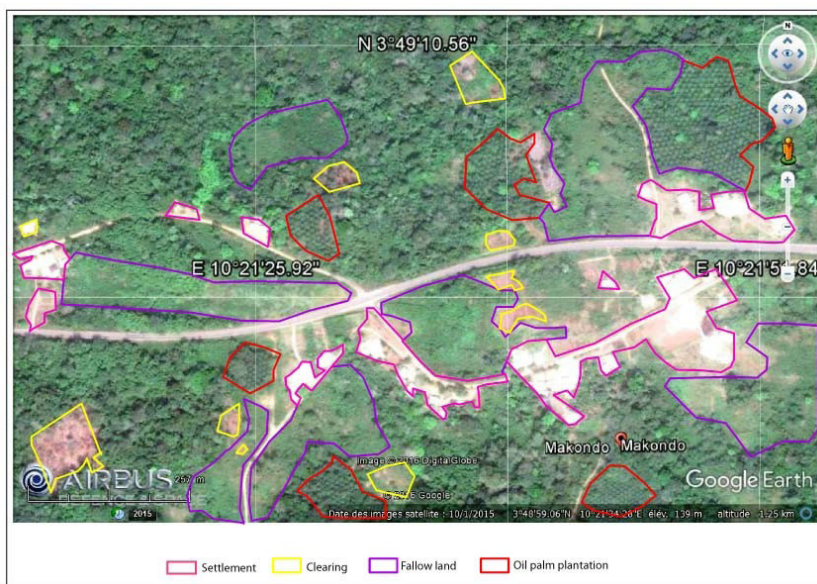
For a mean deforestation of 0.138%/yr (i.e. a loss of 150,000ha of forests/yr), 74% of this deforestation concern areas particularly areas where oil palm agro-industries and small farms are located. Thus, ecology of the oil palm shows that the forest area has the suitable conditions (soil, rainfall, temperature, relief and insolation) for its development. It is in this vein that all old and new palm plantations (from all actors being agro-industries, small farmers and elites) are located in forest areas. This is because forest milieu guarantees them a sustained production over a long period while others areas like fallows and abandoned farms do not bring the expected results (figure 4).

Figure 4: Type of land use chosen for the creation of the oil palm plantations in the main production basins (Source: field survey 2013-2020).



It is worth notice that agro industrial palm plantations / concessions and properties are 100% created on forests. The areas of industrial oil palm producers increased from 46,850 ha in 2009 to 63,200 ha in 2014 and more than 176,600ha in 2019. It means 35% increase in about 5 years and 73% in 10 years. Nevertheless, remote sensing in the Ngwéi and Ekondo-Titi subdivisions as well as Sanaga Maritime Division show that more than 83% of smallholders palm plantations were created to the detriment of primary and secondary forests. The increase is estimated at more than 50% in 10 years between 2000 and 2010. The latter do no longer used fallow land and other abandoned fields. Because there are so many smallholder actors more than 90% for only 15% of area, the accelerated deforestation process by atomizing the forest (figure 5).

Figure 5: Atomization of the forest by unsustainable oil palm worsen practices (construction or building, clearing, palm plantations and wasteland) contributes enormously to the decline of the forest which is suffocated. Any parcel of forest located between two of these plots is doomed to disappearance (Source: map from Google Earth Airbus images).



The threat of palm plantations on the original forest is all the more serious as certain industrial concessions granted in recent years are adjacent to protected areas. This is the case of Sithe Global Sustainable Oils Cameroon (SGSOC); a subsidiary of the American multinational Herakles Farms (Figure 2). The concession acquired by the latter is located in the Kupe-Manenguba division, whose protected areas (namely Korup and Monts Bakossi national parks as well as Rumpi hill reserve and Banyang-Mbo fauna sanctuary) are recognized as High conservation value forests (HCV) and also endemic for its biodiversity. The same situation was observed in the Greenfil case whose palm

plantations are located very close to the Ebo forest which houses wide variety of wild animals, especially the western gorilla, the chimpanzee of Nigeria-Cameroon, the drills and several other primates as well as many endemic plant species. It is the same with the recent de-gazettement in 2019 of 09-025 Forest Management Unit (FMU) to the profit of the installation of new oil palm (60,000 ha) plantation by CAMVERT (Figure 2) at Campo, near the famous Campo Ma'an National park recognized as Model forest and biosphere reserve. The proposed declassified area encompasses two blocks covering 40,000 ha to the north and 20,000 ha to the south bordering Dipikar Island (Campo Ma'an National Park) where there is a gorilla habituation project ongoing.

Deforestation caused through palm planting also breaks up the habitat of endangered species and disturbs wildlife corridors usually used by forest elephants. Such a situation bring confusion and we recorded on fields more and more anger, disappointment and land conflicts due to ambiguous governance of the forest.

### 3.2 THE CHALLENGE OF ECOLOGICAL SUSTAINABILITY

The main biophysical components of environment are exposed to the most worrying negative externalities from the palm oil sector. Based on this ecological pillar, the nature protection organizations continually invite producers and State to put more effort and to pay attention to the following summarized points.

Better integration of oil palm cultivation and pig / poultry farming (plate 1). Pig breeding and poultry farming must be encouraged, because they constitute a better complement and ally of palm farming in terms of waste management and organic fertilization of the fields at the same time as it allows integrated production;

- Integrated management of waste which becomes a source of fertilization and cooking for artisanal oil extraction;
- Avoidance of deforestation and forest degradation by zoning and securing preserved forest areas considered as a land reserve;
- Installation of palm plantations on fallow land (plate 1) and not detrimental to the forest.

Avoidance of the reproduction of cumulative impacts linked to the layering of several activities (construction, artisanal sawing, logging, land clearing, subsistence farming and oil palm cultivation). Finally, the cumulative impacts (physical and human environment) affect the fragmentation of habitats, the loss of biodiversity (see figure 3 above), deforestation coupled with the monoculture of rubber and cocoa or the market food crop including plantain; food insecurity; social conflicts; and finally, social protection and collective bargaining.

**Plate 1:** illustrations of some observed oil palm practices. On the top from left to right: biological fertilization at Bogso; Oil palm plantation on fallow land at Ngwéi; House construction at the entrance to the forest and the palm plantation at Pouma. Bottom from left to right: Last step of oil extraction by artisanal cooking using kernel waste pigs consuming palm nut waste and cake.



As mentioned above, the oil palm is an emerging driver of deforestation since 1990 in Cameroon. Its integration into the process of reducing emissions due to deforestation and forest degradation (REDD +) is a necessity. The resources mobilized by this mechanism can be used to:

- Optimize the use of existing plantations / concessions before considering any form of extension and promote agricultural intensification at the same time with the rehabilitation of old plantations;
- Promote the development of oil palm plantations as a priority in old plantations, abandoned plantations and areas with degraded forest cover.
- Promote sharecropping in the palm oil sector and correctly assess the impacts of investments in the palm oil sector;
- Monitor compliance with land use plans, protection and management of forests in accordance with approved zoning;
- Develop a policy and legal framework for environmental mitigation / carbon offsets of investment projects in forested areas;
- Promote the identification, management and monitoring of areas of high conservation value (HCV) during plantation development.

Equipped with all these assets, the palm oil sector will then present itself as a real opportunity for Cameroon to achieve good performance in REDD +. Rather than creating new plots, it is necessary to favor the restoration of old plantations to

limit the deforestation front. Oil palm, being a forest plantation, can become an asset for the restoration of forests and degraded lands; which would significantly increase the sequestration of greenhouse gases in Cameroon. Once again, the remunerations collected will go into financing the sustainability of this sector.

### 3.3 ELAEISFARMING, EROSION OF BIODIVERSITY AND SOILS CONTAMINATION

The establishment of palm plantations generally involves the almost total clearing of the forest. This leads to the loss of species, including those that are endogenous in certain areas of high conservation value. Oil palm is generally grown in pure culture. It does not tolerate association with other speculation. The other species found in palm plantations are generally the ombrophilous grasses. From our observations, we can affirm that smallholders palm plantations relatively conserve their biodiversity better than industrial ones. This is explained by the solicitation of other ecosystem services such as traditional pharmacopoeia and the harvesting of non-timber forest products by populations.

Oil palm plantations bordered protected areas which is dangerous. Hence the advantage of avoiding installing oil palm plantations next to protected areas or in dense forests because they considerably reduce biodiversity. Better still, in this area, the Government should seek to restore the portions of protected areas eaten away by the palm plantations of agro-industries or elites and other unscrupulous investors.

It is clear that oil palm becomes the source of deforestation and land degradation. Clearing plots during establishment exposes the land to runoff erosion. Certain palm plantations areas such as the district of Ndom and Nyanon have steep slopes, causing the leaching of their soils. The increased use of chemical fertilizers by agro-industries also deteriorates the soil in the long run. In terms of statistics computed since 2013 from processed satellite images, deforestation is estimated at 45.94% in 38 or 40 years, with an overall rate of 697.22 ha / year between 1975 and 2013. Between 1999 and 2013, in 15 years, Ngwéi loose annually 946 ha of dense forest. At this rate, the Ngwéi dense forest may disappear within 50 years (2067) for the normal scenario or within 37 years (2054) if we take the pace of 946 ha / year. In Sanaga Maritime, from 1986 to 2013, deforestation rate is estimated at 23.61%. Figures 6-8 illustrate the smallholders growing scattered and atomize farms in 2013 (Sanaga Maritime division) and 2016 (Ngwéi and Ekondo-Titi Subdivisions) in the studied areas.

Figure 6: Land cover and land use in Sanaga Maritime in 2013 from Landsat ETM+ (P186/r057 & P186/r58) validated with Kappa Coefficient of 95% and after fieldwork. Smallholders' farms are distributed and scattered mostly along the road network.

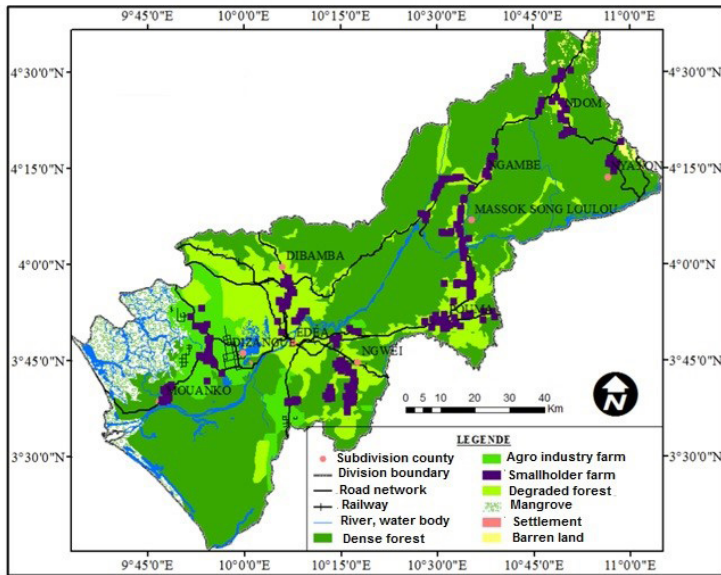


Figure 7: Land cover and land use in Ekondo-Titi in 2016 thanks to Google Earth images (1.5m resolution). We can observe that original forest and mangrove have disappeared everywhere apart from the Northwestern part of the map. Smallholders' farms are spreading northwestern wards.

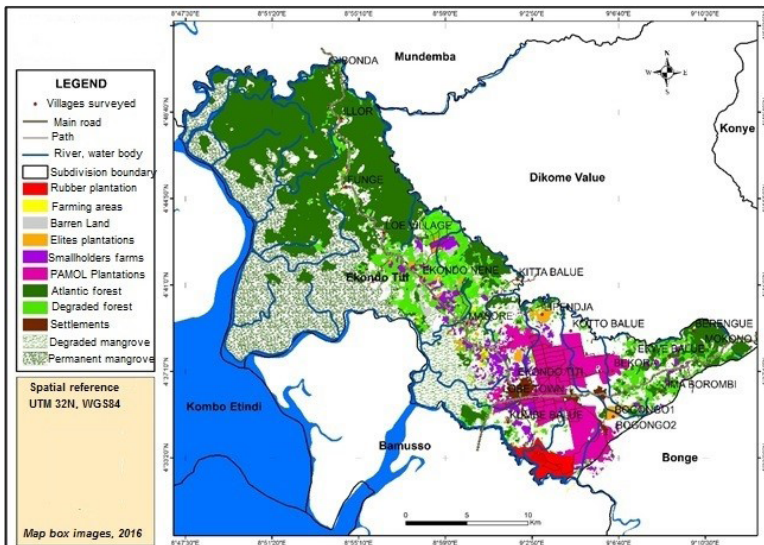
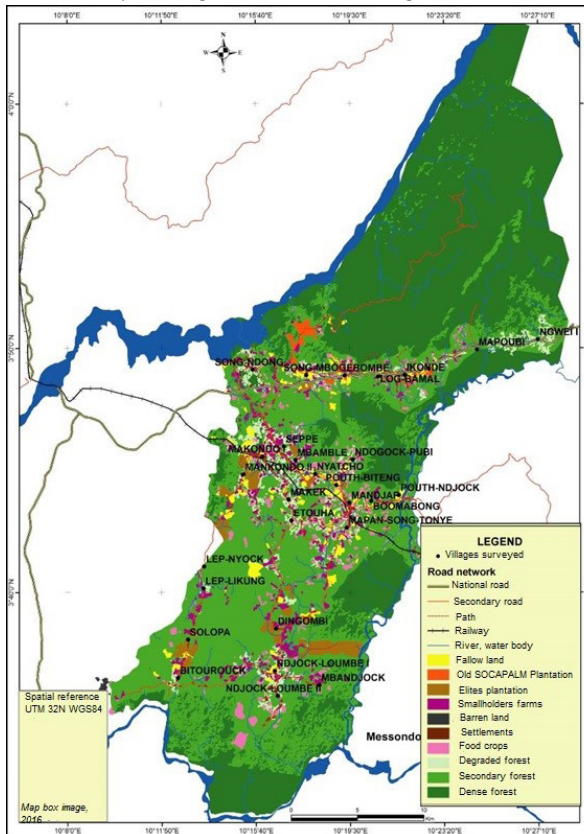


Figure 8: Land cover and land use in Ngwéi District in 2016 from Google Earth images. A small portion southward and a great area northward of the images remain intact and need to be preserved. Numerous smallholders' farms oil palm widespread and scattered in the central part of the image show that the Ngwéi District is the stronghold with almost more than 30% of the areas producing red oil within the Sanaga Maritime Division.



### 3.4 IMPACTS OF ELAEISFARMING IN BIOPHYSICAL MILIEU

Agriculture is the second leading cause of greenhouse gas emissions in Cameroon. It is also the primary driver of changes in land use patterns. Industrial speculations such as cocoa, rubber, sugar cane and oil palm use processes that also damage water causing water pollution. In the context of oil palm, the following stages: clearing for the establishment of new plots, storage of nuts, oil extraction, packaging and waste management are particularly formidable for both air and water. The above table only shows two positive impacts (3.5%) on fauna and NTFPs over 55 negative impacts (96.5%), meaning that oil palm cultivation is disastrous on natural environment. Those impacts are significant for surface water, flora and fauna (biodiversity), soils, natural habitats and NTFPs (Table 5). However, these areas are the most concerned by the safeguard policies of the World Bank and the African Development Bank (ADB), because they are elements of the natural heritage of a country.

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Table 5: Absolute importance of the impacts of the oil palm cultivation on the biophysical milieu.

Component of the affected environment	Activities sources of impacts	Impacts	Characterization parameters and rafting						Final assessment
			Nature	Occurrence	Intensity	Spatial extent	Duration	Reversibility	
Air	Land clearing / deforestation Storage & preparation of nut Oil extraction	Degradation of air quality	•	3	2	1	1	2	1,8
Surface water	Land clearing / deforestation Oil extraction Waste management	Degradation of water quality / contamination, pollution	•	3	3	4	4	4	3,6
Underground water	Land clearing / deforestation Oil extraction Storage & preparation of nut	Contamination, water table attack, pollution	•	3	2	1	3	3	2,4
Soil	Land clearing / deforestation Staking, hole punching Planting Storage & preparation of nut Oil extraction	Degradation of soil quality Contamination, pollution	•	4	4	2	3	4	3,8
Naturel habitat	Land clearing / deforestation Staking, hole punching	Fragmentation, destruction of natural habitats	•	3	3	4	5	5	4
Flora	Land clearing / deforestation Plant maintenance Harvesting bunch Felling old palm plants	Deforestation, fragmentation	•	5	5	4	5	4	4,6
Fauna	Land clearing / deforestation Staking, hole punching Plant maintenance	Fauna habitat disturbance Migration and loss of fauna species	•	4	3	3	5	5	4
Non-timber forest products (NTFPs)	Felling old palm plants	Increase /decrease in NTFPs Loss of medicinal species	•◦	3	2	2	5	5	3,4

Source: fieldwork, 2013-2020.



### 3.5 ELAEISFARMING AND ECONOMIC SUSTAINABILITY

Overall, agriculture contributes 22% of Cameroon’s GDP and employs nearly 62% of the workforce. The oil palm represents 30,000 jobs in the formal sector and at least 50,000 in the informal sector, to which must be added hundreds of thousands of peasants working on their own without being registered (Tchindjang et al, 2016)[23]. Shellfish farming is considered to be a highly intensive labor activity (HILA). In Sanaga Maritime, 51% of the population admits that the oil palm allows them to validly meet their existential needs Ngom et al, 2014[5]. For the elite, this is an area where people invest to earn extra income or prepare for retirement. In the elaeisfarming villages of Cameroon, an abundance of direct or indirect activities linked to this sector makes it possible to more or less effectively rule out the specter of unemployment and poverty. In terms of employment and the local economy, the oil palm despite the importance of negative biophysical impacts keeps the employment side as a positive impact with scores ranking from 3.6 to 4 (Table 6).

Table 6: Absolute importance of impacts of the oil palm on the economy.

Component of the affected environment	Activities sources of impacts	Impacts	Characterization parameters and rafting						Final assessment
			Nature	Occurrence	Intensity	Spatial extent	Duration	Reversibility	
Local economy	Harvesting bunch Packaging and sale	Development of economic activities Increase in income	o	3	3	3	5	4	3,6
Employment	Land clearing / deforestation Staking, hole punching Planting Plants maintenance Storage & preparation of nut Oil extraction Packaging and sale	Job creation	o	4	4	5	4	3	4

Source: fieldwork, 2016-2020.

From an economic standpoint, the benefits of oil palm cultivation are undeniable. This profitability explains the rapid development of the “red gold”. Nevertheless, the contribution of the palm plantation to the local economy and to the well-being of neighboring populations does not always meet expectations. However, in order to strengthen this asset and ensure economic viability, it is necessary to organize and orient the sector in such a way as to ensure a decent income for all stakeholders. The benefits for the

national economy must also be optimized. Palm oil being a source of financial evasion, it is necessary to ensure the autonomy of Cameroon in order, as much as possible, to avoid imports. In practical terms, achieving this goal involves:

- The establishment of an inter-profession between the various actors (agro-industries, elites and smallholders) of the sector to ensure its sustainability and viability.
- Facilitating access to the main inputs / infrastructure (plant material, fertilizers, technical support, roads, etc.) for the benefit of all actors of the palm oil sector.
- Promotion of good management practices for plantations (economic valuation of by-products / by-products, research and use of cheap energy, etc.)
- Promotion of a model of association of agro-industries and surrounding private palm plantations.
- In addition, the development of income-generating activities as petty trade is also observed in these villages thanks to the oil palm cultivation. The same applies to handicrafts made from palm leaves to produce wickerwork products (making brooms, baskets, sieves ... or for roofs). Moreover, palm kernel shells are used in crafts for the manufacture of jewellery (necklaces and others) as well as gadgets for children;
- Lastly, the development of cooperatives based on existing CIGs will constitute the final stage of this economic facet observed in both districts.

Table 7 gathered the perception recorded from farmers and plantations owners about the economic impacts.

Table 7: Perception of livelihood economic impacts through questionnaire and landscape methods.

Area	Sanaga Maritime (n =335)			Ngwéi (n=290)			Ekondo-Titi (n=260)		
	N	No change	P	N	No change	P	N	No change	P
<b>Income level</b>	15	10	75	13	8	79	10	9	81
<b>Quality of social network</b>	25	10	65	27	5	68	15	10	75
<b>Job creation</b>	5	15	85	7	5	88	5	5	90
<b>Social infrastructure</b>	45	35	20	55	25	20	55	30	25
<b>Welfare</b>	10	10	80	5	5	90	4	6	90

N: is Negative P: is Positive.

Better economic results for the oil palm sector in Cameroon also require the establishment of appropriate mechanisms to mobilize resources to finance its development. In terms of economic sustainability, the practice of intercropping (plate 2) is an asset. It is also desirable to diversify economic activities and promote geotopes in elaeisfarming environments with a view to tourism. Finally, improving farmers' incomes and household subsistence is an essential necessity.

Plate 2: Intercropping and oil palm value chain. At the top from left to right is intercropping with banana plantain and maize. Some of the peasants associated also fruits like papaw or coconuts.



Bottom: Oil palm value chain. After cleared old plantations, farmers can extract palm wine (from the trunk) which could be conditioned and sold. Palm wine has also medicinal effect and is used in traditional pharmacopeia and sometimes basket or fly hunting. Palm veins can also be used as brooms after clearing old farms. The last image at the right shows waste packaging from oil extraction to be used as fertilizer or sold.

Food insecurity is caused by low consideration in subsistence or food crops for the benefit of oil palm. This leads to the spectre of famine in a region with such productive lands. Indeed, almost half of the surveyed populations acknowledge that crop yields have declined. The risks of injuries and other accidents are incurred during the clearing, hole digging, cleaning and maintenance of the palm plantation; and above all, oil palm harvesting and the pruning of the palm trees.

### 3.6 SOCIAL ASPECTS OF THE SUSTAINABILITY OF THE OIL PALM SECTOR IN CAMEROON

Social sustainability is quite complex to develop. As we observed on field, it must include social protection, collective bargaining, inclusive dialogue, conflict resolution, corporate social responsibility and environmental justice. These questions variably challenge the agro-industrial and artisanal sub-sectors. In the wake of agro-industrial activities (SOCAPALM, SAFACAM, CDC, PAMOL), we can, more or less, be satisfied with the social protection of employees even if controversies regularly emerge such as the level of wages. The fact remains that the latter are regularly paid and for the most part benefit from some social security. Conversely, almost all of these agro-industrial companies do not adapt well to union activities, especially when they tackle economically sensitive issues such as salary increase, health care, paid leave, security, social benefits of family members of employees, etc. One can add the dictatorship of dismissal which is fashionable and annihilates all prospects for inclusive dialogue and concerted negotiation within companies. It is worth mentioning that the social situation is also tense between the owners of the elite palm plantations and the indigenous populations at Ngwéi. Because the impacts are so important, it is necessary to give sustainable compensation to populations whose land has been occupied by agro-industries in the expected standards.

Over the 83 impacts identified in social environment, 37.35% are positive while 62.65% are negative, signifying that on social domain, oil palm can be seen as a threat. Thus, the social and economic impacts of oil palm cultivation are numerous and sometimes contradictory. It may be overshadowed by the employment and income impacts, but the social consequences of this activity remain numerous.

In the field of the artisanal sub-sector, the face of the sustainability of the elaeisfarming draws a gloomy observation: disorganization of the sector and the market, lack of social security for smallholders, land disputes, conflicts with agro-industries (Table 8), etc. The question is that of a sector that will be fully organized, where the players remain scattered and whose activities sufficiently demonstrate a collective lack of consideration of social sustainability.

Table 8: Absolute importance of impacts of the oil palm on the social environment.

Component of the affected environment	Activities sources of impacts -	Impacts	Characterization parameters						Final assessment
			Nature	Occurrence	Intensity	Spatial extent	Duration	Reversibility	
Conflicts	Plant Care Storage and preparation of palm nuts Fruit bunches harvest Packaging and sales	Land dispute, Aggression, violence Intimidation, threat Tense social climate	•	4	4	1	4	2	3
Human health	Plant Care Storage and preparation of palm nuts	Degradation of workers human health of injury	•	4	3	2	4	2	3
Insecurity	Plant Care Storage and preparation of palm nuts	Injuries Food deficit, social conflicts	•	3	3	2	3	3	2.8
Noise	Storage and preparation of palm nuts	Noise	•	3	2	2	2	1	2
Odour	Storage and preparation of palm nuts Waste management	Degradation of the air quality	•	3	2	2	1	1	1.8
Cultural heritage	Craft production of palm kernel oil	Traditional Pharmacopoeia	o	3	3	5	4	3	3.6
Landscape aesthetics	Creation of new nurseries	Landscape embellishing	o	2	2	1	1	1	1.4

Source: fieldwork, 2013-2020.

Elaeisfarming provides local communities with many materials, social and cultural uses ranging from food to traditional pharmacopoeia through decoration and construction materials, contributing to their well-being and their socio-cultural development. For the traditional pharmacopoeia, palm oil is an antidote to poisons; palm kernel oil is useful for skin care in both new-borns and adults. Lastly, palm wine appears inescapable in all traditional ceremonies and rites concerning enthronement, weddings, deaths and funerals.

Finally, cumulative impacts (physical and human environment) affect habitat fragmentation, degradation and loss of biodiversity, deforestation coupled with the rubber and cocoa single-crop farming or the merchant crop including plantain; food insecurity; social conflicts; social protection and collective bargaining. Table 9 shows the recorded perception of the social impacts by elaeisfarmers.

Table 9: Perception of livelihood social impacts through questionnaire and landscape methods.

Area	Sanaga Maritime (n =335)			Ngwéi (n=290)			Ekondo-Titi (n=260)		
	N	No change	P	N	No change	P	N	No change	P
<b>Access to food</b>	45	20	35	50	20	30	60	15	25
<b>Quality of Housing</b>	12	18	70	10	15	75	20	15	65
<b>Access to land</b>	22	10	68	12	16	72	5	15	80
<b>Access to social infrastructure</b>	50	10	40	50	15	35	40	15	45
<b>Access to drinking water</b>	20	8	72	12	10	78	38	12	50
<b>Water pollution</b>	68	20	12	72	18	10	70	15	15
<b>Insecurity and conflicts</b>	75	10	15	83	10	7	85	5	10

At the socioeconomic level, there are enormous disparities depending on the category of actor; the oil palm value chain seems in fact to benefit more to agro-industrial actors and operators of second and third palm oil transformations. On the contrary, smallholders, because they are not sufficiently taken into account in sectorial policies, are poorly organized, which does not allow them to take the best advantage of the still artisanal oil palm exploitation. The quantitative economic numbers therefore drown the realities.

### 3.7 GOVERNANCE OR POLITICAL SUSTAINABILITY

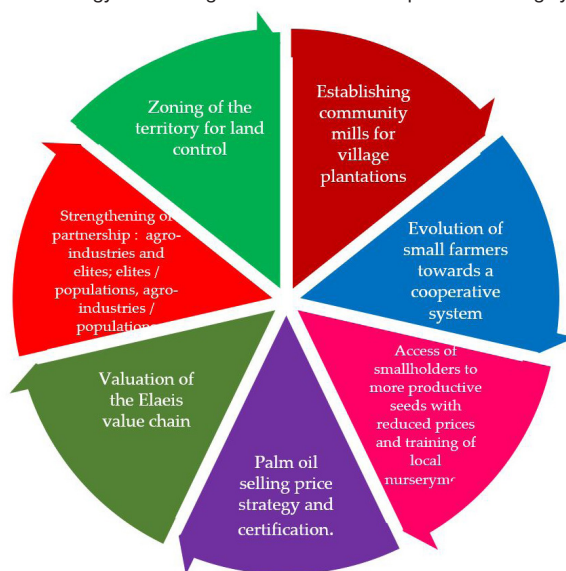
Governance requires having at least a national oil palm strategy, then fighting against deforestation, approving selling prices, rationalizing production and reducing imports. The definition of a strategy and better governance are prerequisites for the sustainability of any economic activity. Cameroon has a national strategy for sustainable development of the palm oil sector which validation is pending. This strategy identifies a set of actors and hierarchical decision-making bodies for the governance of the sector. The national steering committee is responsible for monitoring the implementation of the strategy. To this body, we add programs and projects, professional organizations (inter-professional organizations, cooperatives and unions), consular chambers and national and regional consultation frameworks. Operationally, it becomes urgent to:

- Make a temporal halt in the issue of large concessions for oil palm cultivation and other speculations;
- Prioritize smallholders schemes instead of large scale plantations;

- Apply the same land management requirements to all operators (agro-industry, SMEs) in the land allocation process in order to avoid injustice and deforestation,
- Set up a system for assiduous and regular monitoring of land concessions at the local level;
- Organize and conduct participatory mapping activities of areas and resources (forest) of high conservation value;
- Negotiate, develop and sign the specifications then implement them and make them public;
- Finalize and popularize the zoning plan being developed at MINEPAT for better ownership by stakeholders;
- Develop and set up a system for the redistribution of land royalties in accordance with the regulations in force.

Governance and strategic management are among the axes defined by the Employment Growth Strategy Document (MINEPAT 2009) aiming to make Cameroon an emerging country by 2035. Outside the national framework, good governance must be applied to any economic sector including the oil palm. In addition, securing the elaeisfarming basins prey to attacks by armed groups is seen as a necessity for Cameroon. Figure 9 summarizes the main axes useful for the mainstreaming of sustainability in the oil palm. An integrated and sustainable management approach in the oil palm sector takes into account all stakeholders.

Figure 9: strategy for an integrated and sustainable palm oil farming system.



## 4 DISCUSSION:

### 4.1 ENVIRONMENTAL AND ECOLOGICAL DILEMMA RELATED TO ELAEISCULTURE DEVELOPEMENT

The dilemma arises from the paralleling of the socio-economic advantages and the plural disadvantages that the development of elaeisculture generates in the areas where it is cultivated. In a space with multiple resources and assets, the issues, all of them important, clash and denote the need to delimit the fields of deployment of the different activities. The Sanaga Maritime, Ngwéi and Ekondo Titi are full of very rich forest ecosystems with high conservation value (Table 10). Rainforests sheltering plant and animal biodiversity occupy a large part of the space. The high altitude sectors of the north of the department (Nyanon, Ndom, Ngambé) are home to unique species (endemic and rare). Just like the mangroves of the coastal zone (Mouanko, Dizangué) which constitute the landmark of many animal species in terms of habitat and procreation space. This involves the numerous streams and rivers which structure the very dense hydrographic network of the department.

The issues linked to the preservation of these ecosystems are important, taken in parallel with the pursuit of sustainable development objectives. Better still, this conservation, beyond preserving resources for future generations, contributes to the prospect of developing other economically profitable and ecologically clean activities such as tourism. Political awareness of conservation issues has led to the demarcation of numerous protected areas listed in Table 10 below. By adding up all the categories of protected areas, approximately 25% of natural spaces in these prone areas are classified as protected areas. But, beyond these boundaries, there is a real problem of developing these spaces. Most are abandoned. They have thus increasingly suffered in recent years from attacks by populations who are eating into the outskirts by happily establishing their activities there, particularly palm plantations.

### 4.2 ELAEISCULTURE AND CONSERVATION : AREAS OF ENDEMISM IN CAMEROON

This first diagnosis nevertheless allows us to get an idea of HCV 1 in these territories, particularly around conservation zones and protected areas (PMK, PNDE, PNCM, PNMC) which correspond to the HCV1 category. However, the presence of fresh water and wetlands around Limbe, in the coves of Ndian, Ekondo Titi and especially at Lake Tissongo in the Douala-Edéa National Park allows them to be attributed to HCV4. The threats observed around the PNDE and PNCM make them areas identifiable as HCV3, because we observe a considerable reduction in the surface areas of the ecosystems.



It should be noted that most of these protected areas adjoin UFAs. This is the case of PNCM which surrounds UFA09-020, 09-021, 09-022, 09-023, 09-024 & 09-025 which are all HCV certified forest units. This is also the case for PN Ebo with UFA 07-002. The same applies to PN Korup (UFA 11-002-11-006) PN Bakossi (UFA 11-002) Banyang Mbo (UFA 11-022) etc. Naturally, wetlands recognized as such or associated offer immeasurable ecosystem services. Some are visualized in the images (figures 12 and 13) and may well fit into the components of HCV1.4 and HCV4.

Table 10 :Elæiculture against conservation: Conservation areas of HCV 1 and HCV 4.

HCV identified	Potential or real threats	Objectives	Proposed management measures
<b>HCV 1.1.</b>			
<b>Campo Ma'an NP</b>	Overlapping and nibbling of areas linked to agricultural activities and agro-industries (HEVECAM, SOCAPALM, CDC, PAMOL) Reduction of plant and animal densities on the outskirts Presence of forest concessions Decommissioning of UFA 09-025 next to PNCM Poaching via forest trails or that of agro industries Mangrove degradation	Stabilization or increase in animal and/or plant densities. Adequate and operational zoning of Protected Areas.	Forum or platform for consultation with agro-industries Raising awareness among managers of protected areas and agro-industries Restitution to the conservators of protected areas of areas eaten away by agro-industries Development of corridors for wildlife (especially elephants) Establishment of a regular territorial surveillance system to combat poaching. Blocking and monitoring of access roads and dismantling of bridges after operation. Continue mangrove regeneration activities Restoring forest landscapes
<b>Kribi Marine Park</b>			
<b>Douala-Edéa NP</b>			
<b>Mt Cameroon NP</b>			
<b>Ndongere NP</b>			
<b>Korup NP</b>			
<b>Bakossi NP</b>			
<b>Banyang Mbo Sanctuary</b>			
<b>Ebo NP</b>			
<b>Forest Reserves of Njambo, Yingui, etc</b>			
<b>HVC 1.4 et HCV 4</b>			
<b>Kribi Marine Park</b> <b>Douala-Edéa National Park with Tissongo and Ossa lakes</b>  Places of seasonal concentration of species (marine turtles, manatees, etc.): due to its location or the habitats found there, the concession presents areas of periodic concentration of animals	Deterioration of the state of habitats due to exploitation or other anthropogenic activities Deterioration of the banks of bodies and watercourses Degradation of the habitat bordering the Lakes (Ossa) bodies and watercourses and siltation of water points – Deterioration of junction sites between rivers, bodies of water and roads Exposure of soil on steep slopes and erosion, creating landslides and corollary harmful effects	Safeguard the integrity of these sensitive sites Prevent slopes from being exposed – Avoid obstructing waterways at points	Registration of sensitive sites in the protection series – Materialization of the limits of the most sensitive sites – Implementation of an information and awareness program for local populations on these sensitive sites – Development and implementation of operating procedures avoiding disruption and degradation of identified and recognized sensitive sites stabilization of banks disturbed by engineering structures (anti-erosion devices), compliance with standards regarding slope slopes

Figure 10: Distribution of threatened amphibian species in Cameroon and West Africa (Source: MINEPDED, 2014: 14 and IUCN, 2018: 36).

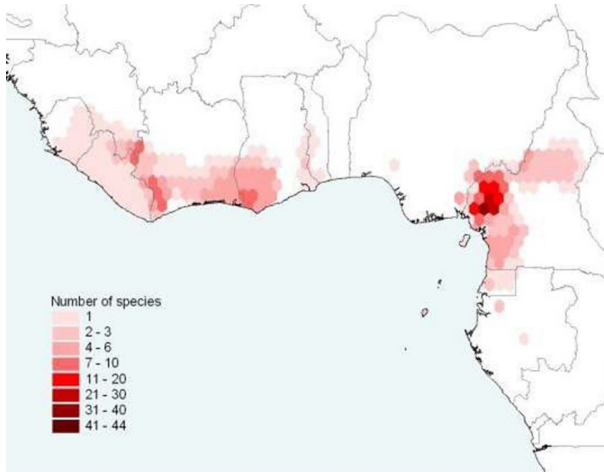
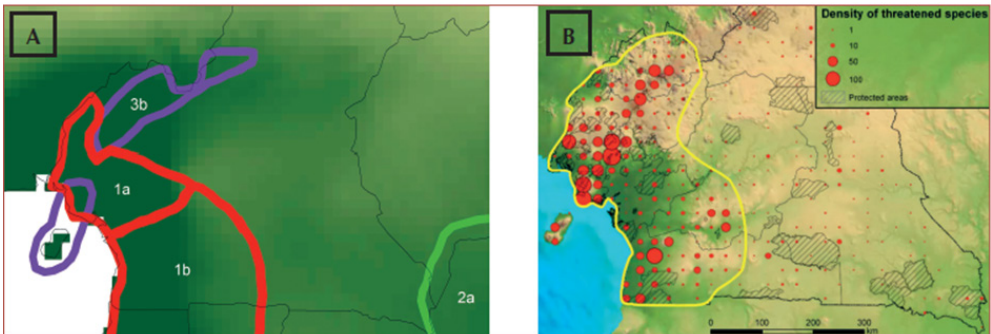


Figure 11: Distribution of threatened amphibian species in Cameroon and West Africa. 2. (Source: MINEPDED, 2014: 14 and IUCN, 2018: 36). 3. Main areas of mammalian endemism, according to OFAC (adapted from <http://www.observatoire-comifac.net>): (1a) West Cameroon region, (1b) Rio Muni region, (2a) West Congo region, and (3b) West Cameroon region. 4. In (B), Density of plant species.



#### 4.3 STRONG DEMAND FOR PALM OIL

As shown before, global demand for palm oil is growing very quickly and supply is struggling to keep pace. Cameroon's national production is also in deficit. Demand is growing rapidly, stimulated by the multiplication of uses of palm oil. Apart from food needs which are growing very quickly, there are those of manufacturers in the field of cosmetics (oil and soap). Also their proximity of the city of Douala (economic capital of Cameroon) reinforces the demand for oil, constantly stimulating the development of elaeiculture. The cosmetics, food and beverage industries and refineries have multiplied, stimulating producers every day to produce a little more. Table 11 below lists the cosmetic industries established on the national territory.

Table 11: Consumption capacities of the cosmetics industries of Cameroon.

Company name		Locatio	Total processing capacity (in ton)	Products	
				Refined oil	Soap
1	AZUR	Douala	15 264	9 994	5 270
2	CCC	Douala	6 552	0	6 552
3	CCO	Bafoussam	3 206	1 690	1 516
4	HACC	Douala	2 496	0	2 496
5	NOSA	Yaoundé	1 197	0	1 197
6	SAAGRY	Douala	2 256	1 632	624
7	SANET	Douala	749	0	749
8	SAS	Douala	1 248	0	1 248
9	SCR MAYA	Douala	15 264	9 994	5 270
10	SCS	Bafoussam	7 264	2 735	4 529
11	SHC	Douala	800	0	800
12	SICT	Douala	En arrêt	/	/
13	SMC	Douala	1 248	0	1 248
14	SMS	Douala	1 089	0	1 089
15	SOC	Bafoussam	1 089	0	1 089
16	SOPROICAM	Douala	1 664	1 664	0
<b>TOTAL</b>			<b>54 242</b>	<b>20 865</b>	<b>33 017</b>

Source: Ngom et al, 2014.

For the moment, public and private initiatives in the oil palm sector have not yet succeeded in curbing needs. At the rate at which the palm oil deficit is evolving in Cameroon, for example, it is clear that oil production has great prospects ahead of it and that the efforts of public and private actors (mainly producers) will have to be doubled in the medium term to satisfy national demand and thus limit imports.

#### 4.4 GOVERNANCE ISSUES

The attitude of the Cameroonian government remains to be questioned in the context of the pursuit of the objectives of sustainable management of natural ecosystems and the development of olive oil production in Cameroon. The government has limited its interventions in the agricultural sector since the economic crisis of the 1990s. This disengagement has contributed greatly to the development of elite palm plantations elsewhere. In the meantime, the population has increased significantly and so has the need for agricultural products. ". Since then, the government project has targeted a growth of 5000ha of planted area per year. In addition, priority was given to foreign private investors to strengthen production and make the country self-sufficient in palm oil production. In

2009, the concession of 73,083 ha of mountain forest with high conservation value to the Herakles Farms group in the South-West region sparked political scandal, provoking the anger of environmental defenders. Increasingly, the government is implementing a land concession policy that is very favorable to industrialists and preferably in sparsely populated areas to limit protests from the population. Which relegates the issues and objectives of conservation of natural ecosystems to second place.

#### 4.5 LESSONS TO LEARN

The ever-increasing development of elaeiculture in the areas studied goes hand in hand with multiple environmental problems. They reveal in space the reverse side of speculation erected as a model of socio-economic development. No! Active elaeiculture here, the underlying levers of an activity which deconstructs existing natural assets and weakens, so to speak, the prospects for sustainable development. In the shadow of its much-touted socioeconomic advantages, it insidiously combines a series of misdeeds that are difficult to repair at the current rate of its growth.

##### ***Deforestation and disappearance of biodiversity***

The development of elastomer production is driving unprecedented deforestation in the Sanaga Maritime, Ngwéi and Ekondo Titi. The illusion of large available land reserves leads elaeiculturists (industrialists, elitists and villagers) to constantly increase the planted areas. The rich forests of those areas are also subject to competition between oil production and subsistence agriculture and logging. They are therefore declining at a very rapid rate (table 12). Since the 2000s, the oil boom has relegated subsistence agriculture and logging far behind and has positioned itself as the main activity driving the deforestation dynamic. Palm groves are created indiscriminately in virgin and secondary forests as well as in fallows left by subsistence agriculture (figure 8a). The losses of biodiversity and multiple resources useful to populations are inestimable.

Table 12: Projected rate of the forest disappearance linked to oil palm cultivation.

Parameters	Sanaga Maritime	Ngwéi	Ekondo-Titi
Area in sq km	9311	848	652
Total deforestation rate	28% in 40 years	45,94% in 40 years	22,74% in 37 years
Mean annual rate	0,7% per year	1,15% per year	0,61% per year
Net deforestation (ha)	121,043ha	11,872	7,882
Deforestation due to oil palm (ha)	65,177	7,632	3,977
Time span projected for the disappearance of the forest	50-70 years	37-50 years	125-189 years
% Oil palm expansion occurring at the expense of the forest	70%	90%	80%

Remote sensing studies of a subset of plantations in 20 countries suggest that about 45% of oil palm plantations in Southeast Asia came from areas that were forests in 1989. Estimates vary by region to the other being 31% in South America, 7% in Africa and 2% in Central America. For Indonesia and Malaysia, the estimates were 54% and 40%, respectively (Meijaard & Sheil, 2019; Meijaard et al, 2020, Sheil et al, 2018). Another estimate gave over the last 40 years, 47% and 16% of total deforestation by oil palm in Malaysia and Indonesia, respectively (Meijaard & al, 2018; Qaim et al, 2020). These statistics can be compared to what we observed in the productive basins of Ngwéi (45.94%) and Ekondo Titi (22.74%) as well as Sanaga maritime (23.61%). According to Ordway et al, (2019), the dynamics of oil palm expansion in sub-Saharan Africa have been neglected. They proved that 67% of oil palm expansion from 2000 to 2015 occurred at the expense of forests in the southwest region of Cameroon.

The replacement of virgin forests by palm monoculture reduces the natural capabilities and capacities of these spaces. Their numerous environmental, social, cultural and economic services are reduced or even disappear.

All around the protected areas, palm groves are taking root and insidiously eating away at the space. The districts of Pouma, Ngwéi and Edéa 1, Ekondo Titi have lost almost all of their virgin forests to the creation of palm groves and more or less subsistence agriculture. The other districts are unfortunately starting this dynamic. Which ultimately questions the proponents of Cameroonian policy in terms of environmental preservation and sustainable development. The increasing deforestation inevitably increases greenhouse gas emissions because it reduces the CO<sub>2</sub> sequestration capacity. The Indonesian example is very illustrative in this regard. The country is home to 3% of the world's forests and ranks 3rd among countries emitting greenhouse gases with a deforestation rate of more than 15%, the highest in the world and mainly due to olive growing. However, Cameroon has joined numerous international initiatives and conventions in this direction: UNFCCC, REDD+, convention on biological diversity, etc.

### ***Soil erosion and impoverishment***

Soil degradation is another major aspect of the development of oil palm growing. The establishment of a palm grove leads to the almost clear cutting of all the trees on the plot. The resulting wood is used for energy purposes. At the very young stage, the surface occupied by the palm grove is at the mercy of erosion. The abundant rainfall in the region makes the phenomenon almost permanent. Despite everything, erosion tends to decrease as the palm trees grow. The widening of the leaflets and their lateral stretching allows the creation of a fairly compact canopy (or roof) which limits the impact of raindrops on the

soil. The palm tree is also a monoculture. Which reflects the fact that it hardly tolerates the presence of other plants. The fact is above all that, the elaeis is very voracious in terms of consumption of organic matter, water and other nutrients from the soil.

### ***Pollution***

It is mainly due to artisanal and archaic palm nut processing techniques and the problem of waste management. If manufacturers argue loudly and to anyone who will listen that their waste is subject to rigorous and ecologically responsible treatment (which at least remains to be verified), village planters who transform their own production with presses craftsmen cannot say the same. The notable fact of this artisanal processing of palm nuts in the is that the artisanal mills are most often located near a watercourse (photo plate 2). Because the palm oil extraction process requires a lot of water.

### ***Poverty of disadvantaged groups***

It is known that there are many socio-economic advantages of elaeiculture: (i) the increase in planters' income; (ii) their Financial autonomy and independence; (iii) the extend of Palm exploitation throughout the year; (iv) the easier managing of the oil stocks; (v) the Self-consumption and marketing; (vi) the exploitation of Many other derivative products; (vii) an embryo of local development.

But, not everyone has the financial means necessary to create a palm plantations. With the development of palm cultivation, it created social classes at the local (village) scale. The owners of palm groves appear more or less as the "well-off" class. Also, people who have not succeeded in olive growing or who live in other activities, particularly subsistence farming, struggle to live decently. In fact, the illusion of "wealth" guaranteed by oil production does not always correspond to the reality on the ground. The cultivation, maintenance and exploitation of palm trees require a lot of financial resources. Very quickly for many people, the plantations are abandoned due to lack of means. In addition, oil production leads to fierce competition for land with subsistence agriculture.

## **4.6 ANY MODEL?**

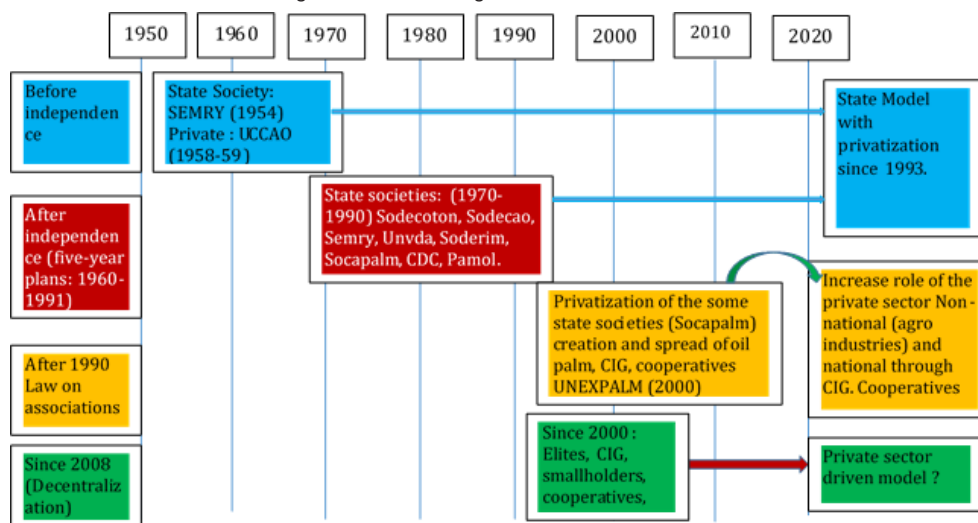
What can be the best model for small holder in Cameroun to boast the red oil production? Is it necessary to gather smallholders in cooperatives or just to provide micro mill per smallholder's community?

The state supports the organization of the sector through production models, which allow small growers to group into cooperatives to benefit from the expertise of the agro-industry. It is a partnership established between cooperatives of smallholder's

farmers, a bank and an agro-industry, all under the supervision of the State. These small planters play an important role in the production of crude palm oil (CPO) because they are more and more numerous and occupy large areas. The total area controlled by small growers increased from 27% in 2007 to 38% in 2011 with 86 million tons of CPO produced (Jan Willem et al, 2013). These models have often been designed to promote rural development, reduce the expenditure of smallholders in transport and fertilizer costs, and provide additional benefits to local communities and migrants. The income is generated by the sale of bunches of fresh nuts (Fresh Fruit Bunches) to the mills, by cooperatives or networks of traders neighboring the plantation (Beekmans et al, 2014).

In Cameroon, agricultural cooperatives have had varying success since the independence era (1960-1970) (figure 12) depending on the crop. The best organized cooperative sector is cocoa and coffee. Cameroon's concern is to strengthen palm oil production in order to meet the country's edible oil demand as well as secondary processing industries. The disorganization between the growers, the non-control of production and the areas of small growers, the import of large quantities of palm oil each year from South-East Asia. The low local production which fails to meet the needs of the populations, as well as the lack of monitoring and orientation of the local activities of small producers which disorganizes the groups, etc.

Figure 12: Cameroon agricultural model since 1950.



## 5 CONCLUSION

The objective of this article was to tackle the dilemma of oil palm cultivation and conservation development in three administrative area Sanaga Maritime Ngwéi and Ekondo

Titi. The results show that this activity is not sustainable and drive deforestation while threatening biodiversity and conservation. It emerges that the oil palm has many negative consequences on the environment such as deforestation and various forms of pollution. Economically, the sector is still dominated by small producers whose methods do not allow satisfactory profitability. In addition, tax collection is lacking, hence the low participation in the national economy. At the social level, wage employment in the field is not well organized and corporate social responsibility is not applied among agro-industries and other large farmers (elites) who nevertheless deserve to be encouraged in this direction if we want to give the local riparian populations the opportunity to benefit from it.

Thus, four levers have been identified to promote sustainability in the palm oil sector in Cameroon: (1) boosting productivity (intensification, best practices and replanting oil farms) to ensure economic profitability; (2) promoting better sharing of benefits; (3) limiting the sector's impact on deforestation and (4) setting up an appropriate framework for good governance in the oil palm sector. This article highlights the actions for each of these objectives for different actors to achieve results. In the economic field, it is necessary to set up self-financing mechanisms for the smallholders' sector and achieve self-sufficiency to avoid imports. In the social field, consultation frameworks must be put in place to promote the consideration of the concerns of all stakeholders, in particular small growers who are harmed and remain the biggest losers in this system. Indeed, already poorly organized, they are not sufficiently taken into account in sectorial policies. With regard to the environment or more specifically ecology, it is necessary to limit deforestation and the pollution induced by the palm oil sector through energetic measures, because we are witnessing a permanent granting of concessions (Greenfil SA in 2014 and CAMVERT in 2019) for oil palm despite warnings and actions from environmental organizations like WWF and Rainforest. Thus, four levers have been identified to promote sustainability in the palm oil sector in Cameroon: (1) boosting productivity (intensification, best practices and replanting oil farms) to ensure economic profitability; (2) promoting better sharing of benefits; (3) limiting the sector's impact on deforestation and (4) setting up an appropriate framework for good governance in the oil palm sector.

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# CAPÍTULO 11

## COMPARACIÓN DE PRUEBAS DIAGNÓSTICAS DIRECTAS PARA LA DETECCIÓN DE *Babesia bigemina* EN BOVINOS<sup>1</sup>

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**RESUMEN:** El diagnóstico específico de la babesiosis, que es causada por el piroplasma *Babesia bovis* y *B. bigemina*, se realiza por la identificación del parásito en frotis sanguíneos teñidos con Giemsa. Aunque el diagnóstico oportuno puede ser realizado por medio de frotis sanguíneos, no siempre puede ser visualizado el parásito en la etapa temprana de la infección. La amplificación de ADN mediante la prueba de PCR (Polymerase Chain Reaction, por sus siglas en inglés) es una rápida técnica que permite incrementar la sensibilidad para la detección de *Babesia* spp. En este trabajo se

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determina el nivel de sensibilidad y especificidad de ambas pruebas, frotis sanguíneo y PCR, para detectar *B. bigemina* durante la fase temprana de la infección. Se utilizaron muestras de sangre congelada de bovinos provenientes de una zona libre de garrapatas y que fueron posteriormente infectados experimentalmente mediante la inoculación de un estabilizado de eritrocitos infectados con *B. bigemina*, el cual había sido mantenido en criopreservación a -196°C en nitrógeno líquido. Se encontró que la prueba de PCR tuvo un 100% de sensibilidad y un 94.44% de especificidad y en el caso del frotis sanguíneo se obtuvo un 100% tanto de sensibilidad como de especificidad. La prueba de PCR para el diagnóstico de *B. bigemina* es lo suficientemente sensible y específica para la detección oportuna de la babesiosis durante su fase temprana.

**PALABRAS CLAVE:** *Babesia bigemina*. Diagnóstico. Frotis. PCR.

## COMPARAÇÃO DE TESTES DIAGNÓSTICOS DIRETOS PARA DETECÇÃO DE *Babesia bigemina* EM BOVINOS

**RESUMO:** O diagnóstico específico da babesiose, causada pelos piroplasmas *Babesia bovis* e *B. bigemina*, é feito pela identificação do parasita em esfregaços de sangue corados com Giemsa. Embora o diagnóstico oportuno possa ser feito através de esfregaços de sangue, o parasita nem sempre pode ser visualizado na fase inicial da infecção. A amplificação do DNA através do teste PCR (Polymerase Chain Reaction) é uma técnica rápida que permite aumentar a sensibilidade para detecção de *Babesia* spp. Neste trabalho é determinado o nível de sensibilidade e especificidade de ambos os testes, esfregaço de sangue e PCR, para detectar *B. bigemina* durante a fase inicial da infecção. Foram utilizadas amostras de sangue congelado de bovinos de uma zona livre de carrapatos que foram posteriormente infectados experimentalmente por inoculação de um estabilizado de eritrócitos infectados com *B. bigemina*, que foi mantido em criopreservação a -196°C em nitrogênio líquido. Verificou-se que o teste PCR apresentou 100% de sensibilidade e 94,44% de especificidade e no caso do esfregaço de sangue foram obtidos 100% tanto de sensibilidade quanto de especificidade. O teste PCR para o diagnóstico de *B. bigemina* é suficientemente sensível e específico para a detecção oportuna da babesiose na sua fase inicial.

**PALAVRAS-CHAVE:** *Babesia bigemina*. Diagnóstico. Frotis. PCR.

## 1 INTRODUCCIÓN

La babesiosis bovina conocida también como fiebre de Texas o fiebre de la garrapata o aguas rojas, es causada por protozoarios intraeritrocíticos del género *Babesia* que son transmitidos por garrapatas (McCosker, 1981). El 70% del total de cabezas de ganado bovino que existe en México, se encuentra en zonas tropicales y subtropicales que son endémicas de la garrapata vector y consecuentemente de la babesiosis (Avila T, 2009).

Existen más de 70 especies de protozoarios del género *Babesia*, distribuidas alrededor del mundo; de las cuales solo 18 causan enfermedad en diferentes mamíferos

domésticos (Levine, 1980). De estas, tres especies destacan por su importancia económica al afectar al ganado bovino (OIE, 2021). Estas especies son: *Babesia bovis*, *Babesia bigemina*, y *Babesia divergens* (Bock et al, 2004; OIE, 2021). En México existen las dos especies de mayor importancia económica *B. bovis* y *B. bigemina* (Toussaint et al, 1905; Alvarez et al, 2019).

Los Médicos Veterinarios buscan el control de enfermedades para mejorar el ganado, sin embargo, la babesiosis es una enfermedad donde el médico encuentra una de sus principales limitantes al no contar con una vacuna comercial (Alvarez, et al, 2020; Bock et al., 2004). Los métodos de control y protocolos de prevención, pretenden hacer más eficientes las condiciones epidemiológicas, sin embargo, las pruebas diagnósticas se han convertido en una herramienta excepcional para el control de las enfermedades parasitarias. La utilización del laboratorio permite conocer los agentes causales, no obstante, muchas de estas pruebas presentan una mayor o menor sensibilidad diagnóstica (OIE, 2023). Debido a esto en este trabajo se pretende determinar el nivel de sensibilidad y especificidad que hay entre dos pruebas directas, el frotis sanguíneo que es una de las técnicas más utilizadas y la Reacción en Cadena de la Polimerasa (PCR, por sus siglas en inglés) que es una de las técnicas de elección ya que se pueden utilizar volúmenes muy reducidos de muestra para detectar la presencia del parásito (OIE, 2021; 2023).

## 1.1 PLANTEAMIENTO DEL PROBLEMA

La babesiosis bovina es la enfermedad protozoaria transmitida por garrapatas que tiene mayor importancia económica en la ganadería de regiones tropicales. Al menos 1.3 billones de animales domésticos están en riesgo de ser infectados, y la mayoría de la población mundial bovina estimada ( $1.2 \times 10^9$ ) está potencialmente expuesta a uno o más especies de *Babesia* sp. (Solorio, 1997).

La amplia distribución geográfica de la babesiosis bovina representa un serio problema para la ganadería, especialmente en los países en desarrollo. Se considera que es un obstáculo especialmente para la introducción de ganado procedente de zonas libre de garrapatas y que es genéticamente especializado en la producción de carne o leche. La presencia de estos parásitos en el ganado es una de las causas más importantes de morbilidad y mortalidad (McCosker, 1981). La población de ganado bovino en México asciende a cerca de 25 millones de cabezas (Avila T., 2009), de las cuales el 70% del inventario nacional de ganado bovino se ubica principalmente en el trópico bajo (Solis, 1991). Esta problemática hace necesaria la implementación de métodos de prevención y de control que deben estar basadas en un sistema de diagnóstico efectivo. Sistema que,

además de indicar la infección individual, permita conocer la situación epidemiológica de la babesiosis en un hato y en una región determinada (Alvarez et al, 2019). Existen distintos tipos de métodos de diagnóstico, una de las formas directas del diagnóstico es la observación del parásito mediante la elaboración y examen microscópico de extendidos sanguíneos teñidos con Giemsa (Solorio et al., 1997). Este es el método más sencillo y económico, con alta especificidad, pero regular sensibilidad, y a pesar de ser útil solo en la fase aguda de la enfermedad el método ha sido, y continúa siendo utilizado como la primera herramienta de trabajo para la identificación y confirmación de los organismos protozoarios (Figuroa et al., 2003).

Las técnicas de tipo indirecto permiten la detección de anticuerpos específicos circulantes (Álvarez et al., 2019). La prueba de inmunofluorescencia indirecta es el método serológico más usado para el diagnóstico de la babesiosis bovina (Solorio et al., 1997; Rojas-Martínez et al, 2018) sin embargo, presenta ciertas desventajas ya que los anticuerpos anti-parásito pueden estar presentes en el suero hasta algún tiempo después de iniciada la infección, o pueden persistir por mucho tiempo posterior a la resolución de la infección, además en los países donde se producen y comercializan vacunas anti-*Babesia*, los animales inmunizados pueden tener anticuerpos que confundirían el diagnóstico, todo esto pudiera ser un problema en términos epidemiológicos (Figuroa et al., 2003).

A diferencia de otros métodos, la prueba de PCR detecta información genética en vez de proteínas, esta técnica es rápida e incluso más sensible que otros métodos, ya que se puede cuantificar la carga viral, bacteriana o parasitaria, y si el producto amplificado es secuenciado, permite investigar la fuente de la infección (Radford, 2009). Sin embargo, su aplicación puede presentar algunos inconvenientes como son los problemas asociados a la contaminación de amplicones (productos ADN de previas amplificaciones), el elevado costo de los materiales y reactivos necesarios, así como los intensos y laboriosos métodos de detección posteriores a la técnica de PCR (análisis de productos amplificados) (Figuroa et al., 2003).

La utilización de la prueba de PCR para diagnóstico, propicia avances significativos no solo en el diagnóstico de la enfermedad, permitiendo diseñar métodos de diagnóstico altamente eficientes, sino también en estudios epidemiológicos (Figuroa et al., 1993, 1998).

## 1.2 HIPÓTESIS

La prueba de reacción en cadena de la polimerasa (PCR) basada en el gen VESA-1 como diana, es más efectiva para detectar la infección por *Babesia bigemina* en sangre de

bovinos y tiene mayor sensibilidad analítica y epidemiológica que el examen microscópico de frotis teñidos. La prueba de PCR basada en la amplificación del gen SBP4 provee una alternativa para el diagnóstico de *B. bigemina* demostrando la misma sensibilidad que la prueba de PCR con el gen VESA-1.

### 1.3 OBJETIVOS

Comparar dos métodos de diagnóstico directo de *B. bigemina* para determinar cuál es más confiable de acuerdo con el nivel de sensibilidad analítica y epidemiológica que ofrece cada uno.

Comparar dos pruebas de PCR con distintos genes blanco de *B. bigemina*, con la finalidad de determinar la sensibilidad analítica en el diagnóstico de *B. bigemina* en animales experimentalmente infectados.

### 1.4 IMPORTANCIA DEL ESTUDIO

La Babesiosis bovina es una enfermedad febril transmitida por garrapatas y es causada por parásitos del género *Babesia* que generalmente se caracteriza por una lisis eritrocítica extensiva que conduce a anemia, ictericia, hemoglobinuria y muerte (Bock et al, 2004). Aunque se han reportado más especies de babesia alrededor del mundo, en Latinoamérica sólo existen dos de ellas: *Babesia bovis* y *Babesia bigemina*. Ambas especies de *Babesia* comparten la garrapata *Rhipicephalus (Boophilus) spp.* como vector para su transmisión biológica al ganado. (Alvarez et al, 2020). En condiciones de campo se presentan regularmente infecciones mixtas con ambas especies de *Babesia* e incluso, asociadas a otras enfermedades como la anaplasmosis. *B. bovis* y *B. bigemina* afectan al ganado de manera distinta y por lo tanto el curso de la enfermedad varía dependiendo de la especie involucrada (Figuroa et al 1993). La importancia de la babesiosis en el trópico mexicano radica en que la infección por este parásito intraeritrocítico provoca grandes pérdidas económicas para los ganaderos, suele ser un obstáculo para mejorar la producción de carne y leche, lo cual exige encontrar un método de diagnóstico efectivo y métodos de control que ayuden a revertir el déficit que México tiene con respecto a importación de leche y carne de origen bovino. Las pérdidas se relacionan directamente con la pérdida de peso en animales enfermos, abortos, gastos por uso de fármacos y atención especializada, además de las pérdidas directas por la muerte de los animales (Alvarez et al., 2019). Además, los animales afectados al recuperarse de la presentación aguda de la enfermedad generalmente se mantienen como portadores asintomáticos por años, pudiendo ser vectores de infección hacia los animales sanos y



difícilmente alcanzan los niveles de producción perdidos (Mahoney, 1994). Actualmente se considera que la babesiosis bovina es una enfermedad que limita la movilización de ganado productor de leche y/o carne, procedente de zonas libres de garrapatas y de *Babesia spp.*, que es trasladado a regiones tropicales y subtropicales. Este tipo de ganado es altamente susceptible a la enfermedad, debido a que nunca ha sido expuesto al vector, ni a *Babesia spp.* (Ross y Mahoney, 1972). Por esta razón es importante la determinación de la estabilidad enzoótica en un rancho o región endémica en particular. Para el diagnóstico de esta enfermedad, se requiere de técnicas que permitan diseñar los procedimientos de prevención y/o controles más adecuados (OIE, 2021; 2023). Rutinariamente se recurre a la observación de las manifestaciones clínicas propias de la babesiosis, además de la identificación de alteraciones patológicas, cambios bioquímicos y evidencias pasadas o presentes de la exposición al agente (Bose et al, 1995). El método más utilizado desde hace años ha sido la identificación de los parásitos babesiales en la sangre periférica en frotis teñidos ya que es un método confiable y rápido, aunque requiere una meticulosa revisión y un microscopista experimentado (Bose et al, 1995; OIE, 2021). Entre las técnicas directas también se encuentran las improntas de órganos, la prueba de hemolinfa y la prueba de PCR. Esta última es una de las técnicas que ofrece importantes ventajas sobre los métodos convencionales de diagnóstico por su alta sensibilidad y especificidad (Figuroa et al., 2003). Los métodos inmunológicos son útiles en estudios epidemiológicos, sin embargo, en situaciones en las cuales se precise de la determinación a nivel especie, y la detección directa del material genético parasitario, es mejor el uso de sondas de ácidos nucleicos o técnicas de amplificación de ácidos nucleicos, tal como la prueba de PCR (Figuroa et al., 2003). Para el diagnóstico de la babesiosis bovina, la implementación de métodos de control y estrategias de prevención, deben estar basadas en un sistema de diagnóstico efectivo, que además de indicar la infección individual establezca las condiciones epidemiológicas de la babesiosis en un hato y en una región determinada. El diagnóstico puede efectuarse de manera directa o indirecta. Una de las formas directas del diagnóstico es la observación del parásito mediante frotis teñidos. Sin embargo, su desventaja radica en que su uso está limitado a la fase aguda de la enfermedad, debido que aun en casos fatales la parasitemia muy pocas veces rebasan el 1% de eritrocitos parasitados. Pese a esto el frotis es una de las técnicas confiables, aunque se requiere un microscopista con un ojo experimentado y una revisión meticulosa; se ha considerado su utilidad en el reconocimiento de ganado portador de *Babesia spp.* (Alvarez M, 2019). Además, gracias a los avances en tecnología molecular se ha hecho posible el uso y análisis de ácidos nucleicos para la detección del

parásito en sangre circulante de bovinos infectados, los métodos más utilizados en el pasado reciente son la hibridación de sondas y la Reacción en cadena de la polimerasa (PCR) (Figueroa, et al, 1993, 2003). Se han diseñado y utilizado un gran número de oligonucleótidos para utilizarse como cebadores en la prueba de PCR para detección de *Babesia* spp (Bock et al, 2004; OIE, 2021). De relevancia y particularmente para el diagnóstico molecular de *B. bigemina* por medio de la prueba de PCR se ha utilizado como diana el gen VESA-1 de *Babesia bigemina*. Brevemente, un fragmento de ADN cortado con enzimas de restricción conteniendo un inserto de 6, 300 pares de bases (pb) de ADN de *B. bigemina* fue subclonado y el extremo carboxilo fue secuenciado por el método de terminación de cadenas mediado por didesoxinucleótidos. A partir de la secuencia obtenida se diseñaron y sintetizaron dos pares de oligonucleótidos (oligos) para ser utilizados en la prueba de PCR. Un primer par de oligos fue utilizado para amplificar un fragmento de 278 pb a partir de ADN genómico del parásito (Figueroa et al., 1992; 2003). Un análisis de homología de secuencia realizado (Ramírez et al, 2011; Pérez et al, 2012) permitió determinar que la secuencia del fragmento de 278 pb es similar a un gene denominado *ves-1* que codifica por un Antígeno Variable de Superficie del Eritrocito (VESA-1 por sus siglas en inglés) identificado originalmente en *Babesia bovis* (O'Connor et al, 1997). VESA-1 es una proteína heterodimérica de alto peso molecular (300 kD) compuesta de VESA1a y VESA1b la cual se sintetiza y posteriormente es transportada a la superficie del eritrocito infectado con *B. bovis*. Los genes que codifican estas proteínas (*ves1*) comprenden la familia más grande de genes (>150 genes) en el genoma de *B. bovis* (Brayton et al, 2007), y genes VESA ortólogos se han identificado en *B. bigemina* (Pérez et al, 2012, Sachman et al, 2021; Santamaria et al, 2024).

## 2 METODOLOGIA

La investigación estuvo dirigida a la comparación de dos pruebas diagnósticas para *Babesia bigemina* (Frotis teñido con colorante de Giemsa y Reacción de la cadena de polimerasa (PCR) para su evaluación como método diagnóstico con mayor sensibilidad y especificidad. También se pretendía establecer que técnica es la más adecuada para detectar la infección durante el periodo de incubación de *Babesia* spp., que tiene una duración de entre 7 y 10 días (Cantó et al., 1999). Además, se comparan la sensibilidad y especificidad de dos pares de oligonucleótidos específicos para 2 genes de *B. bigemina* diferentes: uno donde la molécula diana es un gen ortólogo de VESA-1 (Antígeno Variable de la Superficie del Eritrocito, por sus siglas en inglés) de *B. bovis* y el otro es un gen ortólogo del gen que codifica la proteína SBP-4 de *B. bovis*.

En este caso el primer par, que generalmente es usado para el diagnóstico de *B. bigemina*, ha demostrado ser efectivo en la detección de animales infectados y positivos utilizando la prueba de PCR (Figueroa et al, 1992, 1993). Sin embargo, con respecto al par de oligonucleótidos para amplificar el gen *sbp4*, a la fecha no se ha reportado éste como gen diana para una prueba de PCR, por lo que se pretende sea una alternativa al gen *vesa-1*, donde se espera deberá tener, al menos, la misma sensibilidad analítica de detección que el primero.

## 2.1 GEN *sbp-4* DE *BABESIA BIGEMINA*

Teniendo como base la secuencia del gen que codifica la proteína SBP-4 de *B. bovis* descrita previamente (Terkawi, et al, 2011), se realizó una búsqueda por homología del gen ortólogo de *B. bigemina*, mediante un análisis bioinformático usando la herramienta Blast, en el genoma secuenciado de un aislado Australiano ([http://www.sanger.ac.uk/cgi-bin/blast/submitblast/b\\_bigemina](http://www.sanger.ac.uk/cgi-bin/blast/submitblast/b_bigemina)). El alineamiento de la secuencia *sbp-4* de *B. bovis* con el genoma de *B. bigemina* arrojó dos segmentos de similitud en la secuencia correspondiente a los “contigs” 4098 y 4087. La secuencia codificante de SBP-4 del contig 4098 de *B. bigemina* (834 pb) se utilizó para diseñar 2 pares de oligonucleótidos o iniciadores utilizando la aplicación Primer3 plus disponible en línea (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). Los iniciadores así obtenidos amplificarían un producto esperado de aproximadamente 830 y 740 pb, respectivamente, una vez realizada la prueba de PCR con ADN de *B. bigemina* como molde. Recientemente, se reportó la caracterización del gen *sbp4* presente en *B. bigemina* (Mosqueda et al, 2023) el cual consiste en 834 nucleótidos sin intrones. Este gen se transcribe y se expresa en la etapa eritrocítica del parásito, y se ha demostrado que anticuerpos específicos dirigidos a SBP-4 de *B. bigemina* reaccionan de forma cruzada con *B. ovata*, una especie originalmente aislada de bovinos en Japón, y se propone que estos hallazgos respaldan la inclusión de péptidos específicos de SBP4 en una vacuna contra *B. bigemina* y *B. ovata* (Mosqueda et al, 2023).

## 2.2 BOVINOS

Para esta investigación se utilizaron muestras de sangre congeladas de bovinos provenientes de una zona libre de garrapatas y negativos a anticuerpos contra *Babesia* spp. (por medio de la prueba de inmunofluorescencia indirecta). Los bovinos (n=18) fueron posteriormente infectados experimentalmente mediante la inoculación de un estabilizado

de eritrocitos infectados con *B. bigemina*, mantenido criopreservado a -196°C en nitrógeno líquido (Rojas-Martínez et al, 2018; Santamaria et al, 2020).

### 2.3 DIAGNÓSTICO DIRECTO POR TINCIÓN DE FROTIS DELGADOS

Se realizaron frotis delgados debido a su utilidad en el diagnóstico de babesiosis ya que al extender las células de la sangre en una capa fina se distinguen con mayor facilidad el tamaño de los eritrocitos, las inclusiones y las formas extracelulares (Bose et al, 1995).

### 2.4 DIAGNÓSTICO MOLECULAR: PURIFICACIÓN DE ADN A PARTIR DE MUESTRAS DE SANGRE CONGELADA

Se realizó la purificación de ADN a partir de las muestras de sangre congelada de bovinos experimentalmente infectados (Santamaria et al, 2018), utilizando el kit de Promega (Wizard Genomic DNA purification kit). Se utilizaron muestras de 9 distintas fechas dando un total de 162 muestras congeladas de sangre. Se descongelaron gradualmente las muestras de sangre contenidas en tubos de microcentrifuga de 1.5 ml, posteriormente se colocó la sangre de cada muestra en tubos de 15 ml donde se les agregó solución de lisis (0.015% saponina, 35 mM NaCl y 1mM EDTA) realizando tres lavados por centrifugación con la misma, para eliminar la mayor cantidad de hemoglobina de las muestras, ya que se obtuvo una pastilla de color rosado al fondo del tubo. Se utilizaron 300 µl de cada muestra de acuerdo a las instrucciones del kit Promega (Madison WI, USA) y se colocó en tubos de microcentrifuga de 1.5 ml de nuevo. Posteriormente se continuó con el procedimiento indicado en el kit Promega.

### 2.5 AMPLIFICACIÓN DE LOS GENES ORTÓLOGOS *VESA-1* Y *SBP-4* DE *B. BIGEMINA* MEDIANTE PCR

Las muestras de ADN fueron amplificadas por PCR utilizando únicamente el par de primers (BiF/BiR) para amplificar el gen *vesa-1* (Figueroa et al, 1992; 1993). Para efectos de comparación entre ambos genes, se escogió la fecha pos-infección conteniendo el mayor número de animales con la mayor parasitemia para amplificar por PCR y con los oligonucleótidos para el gen *sbp-4*. Partiendo de ADN purificado se llevó a cabo la amplificación de los genes *vesa-1* y *sbp-4* para la detección de *B. bigemina* mediante la reacción en cadena de la polimerasa (PCR) con un volumen final de 25 µl, donde se incluye 12.5 µl de mezcla maestra (Promega, Co.) conteniendo Taq polimerasa, dNTPS, Cloruro de Magnesio, 1 µl de cada uno de los oligonucleótidos para el gen *vesa-1* (BiF,

5´-TCTCTCCATACCCCTCCAAC-3´ y BiR, 5´GCTTCAACTCTGAGTCCAAA-3´) o *sbp-4* (F, 5´-AATGTCATCGAGGTGACCTTCC-3´, y R, 5´CTGGTGCTCCTCAACCTCCTT-3´); 2.5 µl de agua destilada y 8 µl de ADN purificado. La amplificación se llevó a cabo en un termociclador Biorad bajo el siguiente programa de ciclado de alineamiento a: desnaturalización inicial 95°C por 5 min., desnaturalización a 95°C por 1 min., alineamiento a 57°C por 1 min., extensión a 73°C por 1.5 min durante 35 ciclos y extensión final por 72°C por 15 min., y para SBP-4: desnaturalización inicial a 95°C por 5 min., desnaturalización a 94°C por 30 seg., alineamiento a 61°C por 90 seg., extensión a 72°C por 2 min durante 45 ciclos, finalizando con un ciclo de 72°C por 15 min.

## 2.6 VISUALIZACIÓN DEL PRODUCTO DE PCR

El producto de PCR se analizó mediante electroforesis horizontal en un gel de agarosa al 2% en buffer TAE 1X, teñido con 2 µl de bromuro de etidio (10 mg/ml). Se utilizaron como marcadores de talla las escaleras de 100 y 50 pb y, en algunas ocasiones, de 1 Kb.

## 2.7 ANÁLISIS ESTADÍSTICO

Para identificar relaciones de dependencia entre variables cuantitativas se utilizó la prueba de  $\chi^2$  (Statistix 9), teniendo significancia los valores  $p < 0.05$ .

## 2.8 CRITERIOS DE FIABILIDAD

Para determinar los criterios de fiabilidad, se realizaron tablas de doble entrada, donde se compararon los resultados obtenidos por microscopía de frotis sanguíneo vs los obtenidos mediante la prueba de PCR. La detección directa por microscopía fue considerada como la técnica "gold standard", dado que es la tradicionalmente utilizada en el diagnóstico de una infección por *Babesia* spp (Figuroa et al 2003; Calder et al, 1996). Se evaluó el desempeño de las pruebas en términos de sensibilidad, especificidad, valor predictivo positivo (VPP), valor predictivo negativo (VPN). Para estimar la concordancia entre la prueba de PCR y Frotis sanguíneos se determinó el valor del coeficiente de kappa con intervalo de confianza del 95 % (IC).

## 3 RESULTADOS Y DISCUSION

### 3.1 COMPARACIÓN ENTRE MICROSCOPIA ÓPTICA Y PRUEBA DE PCR

Se corroboró la presencia de parásitos intraeritrocíticos en los bovinos a partir del 5º día pos-inoculación (DPI) mediante la observación al microscopio de frotis sanguíneos.

En el cuadro 1 se muestra el porcentaje de parasitemia que se obtuvo individualmente en cada día pos-inoculación y el promedio por día, donde se puede apreciar que las mayores parasitemias se obtuvieron en los días 7, 8 y 9 PI.

Cuadro 1. Porcentaje de parasitemia y promedio por día Pos-Inoculación (DPI).

ID \ DPI	5	6	7	8	9	10	16
6	0.005	0.02	0.255	0.15	0.22	0.1	0
7	0.005	0.015	0.105	0.125	0.23	0.1	0
9	0.01	0.035	0.035	0.045	0.009	0.06	0
10	0.02	0.035	0.05	0.04	0.28	0.0318	0
12	0.01	0.075	0.095	0.125	0.04	0.05	0
13	0.005	0.02	0.075	0.075	0.025	0.11	0.01
15	0.005	0.01	0.155	0.175	0.02	0.01	0
19	0	0.005	0.01	0.01	0.039	0.005	0.01
20	0	0.015	0.015	0.1	0.02	0.005	0.015
21	0.005	0.08	0.225	0.06	0.079	0.045	0.015
22	0.01	0.04	0.08	0.075	0.075	0.01	0.005
23	0.05	0.15	0.15	0.5	0.25	0.25	0.005
25	0.005	0.005	0.14	0.015	0.25	0.05	0
28	0	0.02	0.02	0.15	0.45	0.12	0
41	0.005	0.01	0.015	0.05	0.4	0.08	0
45	0	0.005	0.05	0.15	0.054	0.008	0
46	0	0.01	0.038	0.105	0.17	0.145	0
112	0	0.01	0.115	0.02	0.05	0.005	0.01
<b>Promedio</b>	0.009	0.031	0.109	0.109	0.147	0.066	0.004

Se procesaron 126 muestras utilizando la prueba de PCR-vesa-1. Los resultados globales se expresan en el cuadro 2, donde se muestra el número de animales positivos por la prueba de PCR a partir del 5° día pos-inoculación (DPI).

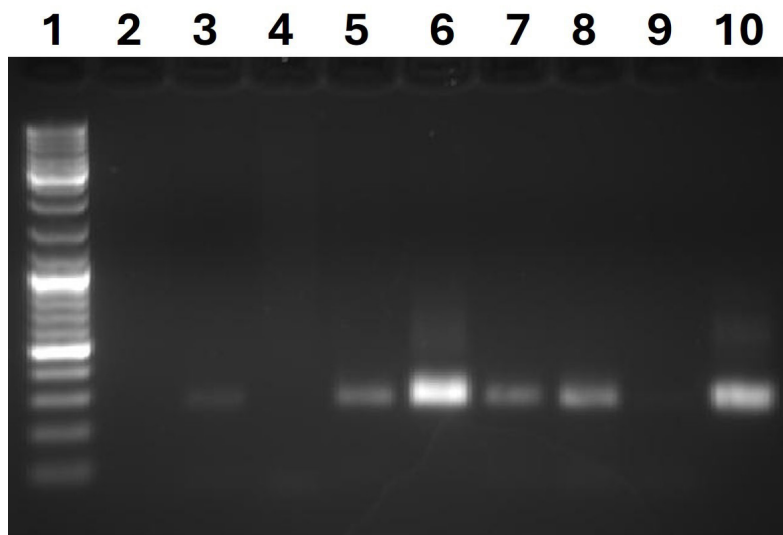
Cuadro 2. Resultados de la prueba de PCR-vesa-1.

ID \ DPI	5	6	7	8	9	10	16
6	-	-	+	+	+	+	+
7	-	-	+	+	+	-	+
9	-	-	+	+	+	+	+
10	-	+	+	+	+	+	+

<b>12</b>	-	+	+	+	+	-	+
<b>13</b>	-	-	+	+	+	+	+
<b>15</b>	-	-	+	+	+	-	-
<b>19</b>	-	-	+	+	+	+	+
<b>20</b>	-	-	+	+	+	+	+
<b>21</b>	-	+	+	+		+	-
<b>22</b>	-	+	+	+	+	+	-
<b>23</b>	-	-	+	+	-	-	+
<b>25</b>	-	-	-	+	+	-	-
<b>28</b>	-	-	+	+	+	+	-
<b>41</b>	-	+	+	+	-	-	-
<b>45</b>	-	+	+	+	+	-	-
<b>46</b>	+	-	+	+	+	+	+
<b>112</b>	-	-	+	+	+	+	+
<b>No. de bovinos +</b>	1	6	17	18	16	11	11

En todos los casos que hubo amplificación, se observó una banda única de aproximadamente 270 pares de bases, de manera similar a lo obtenido por Figueroa *et al.* (1992; 1993). En la Figura 1 presentan los resultados representativos del seguimiento realizado en 1 bovino.

Figura 1.- Análisis de la amplificación por PCR-*vesa-1* de muestras del Bovino número 46. Gel de agarosa al 2% teñido con 0.2% de bromuro de etidio, visualizado con luz UV. Identificación de carriles: 1) Marcador de 100 pb; 2) Día Pos-Inoculación (DPI) 0; 3) DPI 5; 4) DPI 6; 5) DPI 7; 6) DPI 8; 7) DPI 9; 8) DPI 10; 9) Control PCR negativo; 10) Control PCR positivo.



### 3.2 COMPARACIÓN DE LAS 2 TÉCNICAS DE DIAGNÓSTICO: MICROSCOPIA DE FROTIS SANGUÍNEO Y PRUEBA DE PCR

Al comparar los resultados obtenidos mediante la prueba de PCR con los de microscopía de frotis sanguíneo, se obtuvo que, de 126 muestras analizadas, 73 fueron diagnosticadas como positivas a *B. bigemina* por ambas técnicas. Los animales que fueron positivos por microscopía de frotis sanguíneos se encontraban, al parecer, en un pico de la parasitemia, que hace que los niveles del parásito aumenten y puedan ser visualizados al microscopio, teniendo en cuenta la relativamente baja sensibilidad de la técnica (Bose et al, 1995; Calder et al, 1996). Las 53 muestras restantes difieren en su diagnóstico, pues 7 de las muestras resultaron positivas por PCR y negativas a la microscopía de frotis sanguíneo, y las 36 muestras restantes fueron negativas por PCR y positivas por microscopía de frotis sanguíneo.

En el primer caso, podría deberse a que estos bovinos presentaron porcentajes muy bajos de glóbulos rojos infectados para poder ser detectados mediante el examen por microscopía de frotis sanguíneo durante al menos 5 min. En el segundo caso, en el que las muestras negativas por PCR fueron positivas a microscopía de frotis sanguíneo, pudiera deberse a que el porcentaje de parasitemia que se reportó pudiera tener falsos positivos ya que en climas húmedos es común que el tiempo de secado del frotis sea



más prolongado y esto ocasiona que se formen burbujas que pueden aparentar formas inmaduras de *Babesia*. Alternativamente, y dado que los frotis fueron evaluados por microscopistas expertos, es probable que el nivel de sensibilidad analítica de la prueba de PCR sencilla no sea lo suficientemente elevado como para detectar parasitemias bajas. Si bien todos los animales inoculados fueron detectados positivos al examen microscópico, las parasitemias determinadas fueron muy bajas comparadas con los resultados obtenidos en otros experimentos (Figuroa et al, 1998; 2003; 2006). Esto, muy probablemente deriva del hecho que, en este experimento en particular, los animales fueron inoculados con un estabilizado criopreservado en nitrógeno líquido, pudiendo verse afectado el título de parásitos viables e infectantes una vez descongelado el material previo a su inoculación. Sin embargo, en general, el número de veces que la microscopía óptica de frotis detectó positivos fue siempre mayor que las muestras determinadas por PCR durante los primeros 10 días pos-inoculación. Se realizó una comparación entre las dos técnicas de diagnóstico, por día, y a partir del 5º DPI. Utilizando el método estadístico  $\chi^2$  se verificó si había diferencia significativa entre el número de positivos obtenidos con cada una de las técnicas, en el cuadro 3 se observa el porcentaje de positivos y su comparación.

Cuadro 3. Comparación del número (y porcentaje) de muestras positivas con la prueba de PCR y observación microscópica de frotis sanguíneo.

Prueba	DPI 5	6	7	8	9	10	16
<b>PCR</b>	1 (5.5%) a	6 (33.3%) a	17(94.4%) a	18(100%) a	16(94.1%) a	11(61.1%) a	11(61.1%) a
<b>Frotis</b>	12(66%) b	18(100%) b	18(100%) a	18(100%) a	18(100%) a	18(100%) a	7 (38.8%) a

Literales distintas en cada columna representan diferencia significativa ( $P < 0.05$ )

Se pudo establecer que a los cinco días pos-inoculación el frotis sanguíneo presentó un 66.6% de positivos (en comparación con PCR que mostró 5.5%,  $p = 0.0005$ ), a los seis días pos-inoculación la microscopía detectó el 100% de positivos, mientras que la prueba de PCR solamente detectó un 33.3% de positividad ( $p = <0.001$ ) lo que indica que hubo una diferencia significativa entre el diagnóstico por frotis sanguíneos y PCR para estos dos días en particular. Se demuestra así, que el número de animales detectados como positivos es dependiente de la técnica diagnóstica utilizada. Sin embargo, esto difiere de los resultados encontrados por Krause et al. (1996) donde se realizó la comparación entre las mismas técnicas en *B. microti* y en cuyo caso se

encontró que la prueba de PCR obtuvo un mayor número de resultados positivos en comparación al examen microscópico de los frotis. No obstante, este resultado puede deberse también, a que el estudio fue realizado durante la fase aguda de la enfermedad, período en el cual las parasitemias fluctúan constantemente, independientemente de la variabilidad a infección, innata o adquirida, observada en los animales experimentalmente infectados (Allred, 2007).

Se realizó adicionalmente la comparación de los resultados un día posterior al tiempo de incubación, escogido al azar (día 16 PI), en donde se observó que la prueba de PCR detectó un mayor número de positivos y aunque no hubo diferencia significativa ( $p > 0.05$ ) el resultado coincide con los resultados obtenidos por Figueroa (1992), en donde a pesar de las bajas parasitemias ( $<0.01\%$ ) se detectaba un mayor número de positivos a PCR que a microscopía óptica. Se puede especular entonces que, en el tiempo posterior a la incubación, una vez transcurrida la parte inicial de la fase aguda de la enfermedad, la prueba de PCR puede detectar un mayor número de animales positivos a *B. bigemina*.

Por lo tanto, y de acuerdo con los resultados anteriores, el examen microscópico de los frotis sanguíneos tiene mayor sensibilidad analítica para detectar la infección en la fase temprana de la enfermedad, por lo menos a nivel experimental. Esto coincide con otros estudios, en donde se detectan animales positivos por primera vez hasta el décimo día PI y con parasitemias de 0.014 y 0.038%. En cambio, en el caso de los días posteriores, la técnica de PCR detectó mayor número de animales positivos en comparación con el examen microscópico de los frotis, cuando se determinaron parasitemias de  $\leq 0.01\%$ , consideradas como bajas (Figueroa et al., 1992).

En estudios anteriores se ha determinado que la prueba de PCR asociada a la detección del producto amplificado con una sonda colorimétrica específica puede detectar hasta una parasitemia de 0.00001% (Figueroa y Álvarez, 2003; Figueroa et al., 1993). Sin embargo, en el presente estudio, en el que solo se analiza el resultado de PCR sin hibridación con sonda específica se pudo observar que cuando se determina una parasitemia promedio de 0.00861%, sólo el 5.5% de los animales probados es detectado por la prueba de PCR sencilla. En contraste, cuando las parasitemias son del orden de 0.031% la prueba de PCR detecta un 33.3% de animales positivos, mientras que con parasitemias relativamente más elevadas (de 0.065%) se detecta hasta el 61.1% de animales positivos en la prueba de PCR sencilla. A diferencia de lo anterior, cuando se analiza el período comprendido entre el 7º y 9º día pos-inoculación, se pudo determinar que la técnica de PCR tiene un 100% de capacidad para detectar los animales infectados verdaderos positivos (con parasitemias estimadas entre 0.109 y 0.147%); y a los 16 días

PI con una parasitemia promedio de 0.0038% se detectó un mayor número de positivos, aunque no hubo diferencia significativa ( $p = 0.182$ ).

### 3.3 DETERMINACIÓN DE CRITERIOS DE FIABILIDAD

Se determinaron las características de las pruebas mediante un cuadro de contingencia de 2x2. Como muestras negativas, se utilizaron las muestras obtenidas en la fecha previa a la inoculación experimental en donde los animales muestreados eran negativos a la infección.

Cuadro 4. Comparación de la prueba de PCR con frotis delgados teñidos con Giemsa para 18 muestras positivas y 18 negativas a infección por *B. bigemina*.

Prueba diagnóstica	Sensibilidad (%)	Especificidad (%)	Valor predictivo positivo (%)	Valor predictivo negativo (%)
PCR	100	94.44	94.7	100
Microscopía de Frotis	100	100	100	100

En cuanto a los criterios de fiabilidad (Cuadro 4), la técnica de microscopía de frotis sanguíneo presentó sensibilidad y especificidad de 100%, valor predictivo positivo y valor predictivo negativo de 100%, es decir, utilizando esta técnica se clasificaron correctamente como enfermos al 100% de los que padecieron babesiosis y como sanos al 100% de los que no se encontraban enfermos de babesiosis. En cuanto a los valores predictivos, ellos significan que en un 100% de los bovinos con babesiosis finalmente se confirmó la presencia de *B. bigemina*, mientras que en los animales negativos (muestreados previamente a la inoculación) en los que no se detectó la presencia de la enfermedad un 100% estaba efectivamente sanos. Estos resultados no difieren mucho con los resultados reportados por Figueroa *et al.* (1996), donde reporta valores de sensibilidad y especificidad de 93,8 y 100%, respectivamente y valores predictivos positivo y negativo de 100% y 97%, respectivamente.

La microscopía de frotis sanguíneo con tinción de Giemsa es el método más común para identificar *Babesia spp.* en animales con infección clínica. Esta técnica permite diagnosticar principalmente a los animales en fase aguda (Bose *et al* 1995; Figueroa & Buening, 1995). La prueba de PCR es una técnica molecular de comprobada elevada sensibilidad y especificidad (Calder *et al.*, 1996). En el cuadro 4 se muestra que se obtuvo un 100% de sensibilidad y un 94.44% de especificidad, el valor predictivo positivo fue de 94.7% y el valor predictivo negativo de 100%, es decir, utilizando esta técnica se clasificaron correctamente como enfermos al 94.4% de los que padecieron babesiosis y

como sanos al 100% de los que no se encontraban enfermos de babesiosis. En cuanto a los valores predictivos, ellos significan que en un 94.7% de los bovinos con babesiosis finalmente se confirmó la presencia de *B. bigemina*, mientras que los animales en los que no se detectó la presencia de la enfermedad, un 100% estaba efectivamente sanos. Estos resultados difieren ligeramente con los resultados reportados por Krause *et al* (1996), donde reporta valores de sensibilidad y especificidad de 95% y 100%, respectivamente, y valores predictivos positivo y negativo de 100% y 83%, respectivamente, para el diagnóstico de *B. microti*. Estos resultados permiten establecer que la prueba de PCR, con los oligonucleótidos específicos de gene y especie, es una técnica aceptable para el diagnóstico de *Babesia spp.*, y no solo de *B. bigemina*. Con los resultados de ambas técnicas diagnósticas se determinó un coeficiente de concordancia de 0.97 que lo ubican como muy bueno, esto puede interpretarse como que ambas pruebas tienen una utilidad similar para efectos de diagnóstico de *B. bigemina* en los casos agudos de la enfermedad.

No obstante que la prueba de PCR presenta una sensibilidad y especificidad similar a la de la prueba estándar de oro (Microscopía óptica), el examen de frotis demostró identificar un mayor número de animales positivos verdaderamente positivos durante la incubación de la enfermedad. Sin embargo, es importante enfatizar que esta prueba requiere de un microscopista experimentado, de lo contrario podrían identificarse algunos casos como falsos positivos, pero más importante aún, la inadecuada identificación de formas o estadios del parásito (no clásicas para un microscopista carente de experiencia), podría dar como resultado la asignación de falsos negativos, con la consecuente falta de oportunidad para un tratamiento adecuado de un animal infectado. Se ha cuestionado el costo elevado de la prueba de PCR para uso rutinario en un laboratorio de diagnóstico, haciendo todavía más viable el uso del frotis sanguíneo para el diagnóstico de babesiosis causada por *B. bigemina* durante las etapas tempranas de la enfermedad. Sin embargo, en la medida en que se abaten los costos de los materiales y reactivos para la prueba de PCR, ésta es una técnica que bien podrá utilizarse como complemento de diagnóstico en el caso de animales con casos agudos y más importante, casos subclínicos, los cuales, por estar en la fase aguda, pero sin manifestación abierta de signos clínicos de enfermedad, podrían dar falsos negativos debido a la fluctuación de las parasitemias que se reportan durante esta etapa (Calder *et al.*, 1996). Además, la prueba PCR puede ser recomendada para determinar la presencia del parásito en animales de importancia reproductiva como sementales y animales donadores de embriones, cuando se requiera el traslado de los mismos, y también puede ser de gran auxilio en los monitoreos de programas de

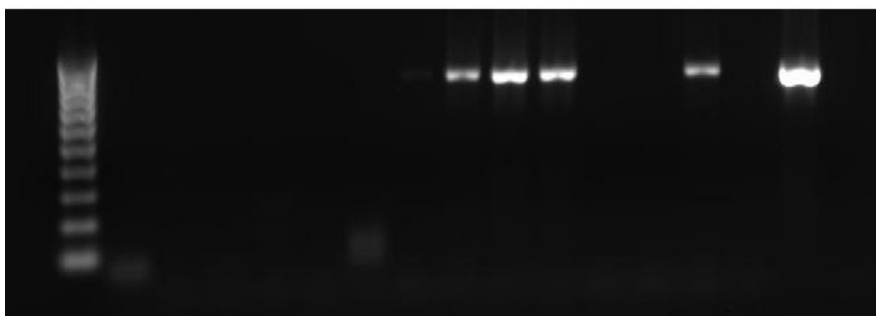
lucha integrada de garrapatas y enfermedades asociadas. Sin embargo, y a pesar del relativamente elevado costo, el uso de la prueba de PCR resulta particularmente apropiada y especialmente se aplica en proyectos de investigación para el desarrollo de estudios epidemiológicos a gran escala (OIE, 2021; Ganzinelli et al, 2020). Esto, derivado del hecho de que, con los nuevos procedimientos y reactivos comercialmente disponibles, se pueden procesar literalmente cientos, si no miles, de muestras sanguíneas en un solo día. A diferencia de la microscopía óptica, en la que un experto microscopista puede solo analizar, objetiva y concienzudamente, una treintena de frotis sanguíneos en una jornada (Figuroa et al, 1996).

### 3.4 COMPARACIÓN ENTRE LAS PRUEBAS DE PCR CON OLIGONUCLEÓTIDOS PARA AMPLIFICAR *SBP-4* O *VESA-1*

Amplificación del gen *sbp-4*: Mediante la técnica de PCR se amplificaron fragmentos de la región codificante del gen *sbp-4* de *B. bigemina*, obteniéndose un amplicón del tamaño esperado, aproximadamente 830 pb (Figura 2). La prueba de PCR para el gen *sbp-4*, permite identificar infección en animales probados a partir del día 10 PI (ver resultado representativo, Figura 2).

Figura 2: Análisis de la amplificación por PCR-*sbp-4* de muestras del Bovino 23: Gel de agarosa al 2% teñido con 0.2% de bromuro de etidio, visualizado con luz UV. Identificación de carriles: 1) Marcador de 100 pb; 2 - 14) Día Pos-Inoculación (DPI) 0 - 13; 15) Control PCR negativo; 16) Control PCR positivo.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



Se comparó una fecha pos-inoculación seleccionada al azar (Cuadro 5) con ambas pruebas de PCR dando como resultado para el gen *vesa-1* un total de 14 positivos de 17 muestras analizadas, mientras que en el caso del gen *sbp-4* se obtuvieron 15 positivos. Probablemente la capacidad del PCR-*sbp-4* para detectar un mayor número de positivos que el PCR-*vesa-1* se deba a que el primero tiene un ciclo de 45 repeticiones de ciclado, por lo que puede realizar un mayor número de copias del ADN del parásito.

Cuadro 5: Resultados de PCR con los genes *sbp-4* y *vesa-1* de muestras del día 9 PI.

PCR / ID	6	7	9	10	12	13	15	19	20	22	23	25	28	41	45	46	112
<i>vesa-1</i>	+	+	+	-	+	+	+	+	+	+	-	+	+	-	+	+	+
<i>sbp-4</i>	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+

## 4 CONCLUSIONES

Las pruebas diagnósticas utilizadas para identificar *B. bigemina* tienen características particulares, dentro de estas características cabe mencionar el funcionamiento, la sensibilidad, la complejidad y el costo de cada una. Las ventajas y desventajas de cada prueba, principalmente en lo que se refiere a los criterios de fiabilidad, deben tomarse en cuenta al momento de seleccionar una técnica diagnóstica para uso rutinario. El objetivo general de esta investigación fue el de evaluar que técnicas diagnósticas son más convenientes para el diagnóstico de *B. bigemina*. La técnica de microscopía de frotis sanguíneo, por su valor predictivo positivo alto, se puede usar para confirmar la presencia de *Babesia*, cuando no se disponga de técnicas de alta sensibilidad y especificidad como son la PCR, y cuando se trate particularmente de casos agudos de la enfermedad. Se detectó una diferencia significativa entre el diagnóstico por frotis sanguíneos y PCR para los días cinco y seis pos-infección, por lo cual se podría suponer que la técnica utilizada sí influye en la detección de la presencia o ausencia de la enfermedad en los bovinos muestreados. La prueba de PCR-*sbp-4* dio un mayor número de resultados positivos que la prueba de PCR-*vesa-1*. La prueba de PCR podría ser usada como prueba tamiz en trabajos epidemiológicos debido a su alta sensibilidad y especificidad, aunque presenta un relativamente alto costo.

**Recomendaciones:** Comparar por un periodo de tiempo más prolongado (fase aguda y crónica) la sensibilidad y especificidad de la prueba de PCR y la microscopía de frotis. Realizar una comparación entre las pruebas de PCR-*vesa1* con PCR-*sbp-4* pero aumentando el número de ciclos para el gen *vesa1* para observar si es posible incrementar el nivel de sensibilidad sin necesidad de un PCR adicional o anidado. Por otro lado, futuros trabajos deben realizar la misma comparación, pero con PCR anidado o PCR en tiempo real para ambos genes, y con muestras de bovinos inoculados experimentalmente y en bovinos infectados naturalmente, para estimar su verdadero potencial diagnóstico, determinando el porcentaje de sensibilidad y especificidad de cada prueba. Utilizar la técnica de PCR anidado como una técnica de diagnóstico al adquirir animales para experimentación que requieran estar negativos a *Babesia spp.*

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# CAPÍTULO 12

## OZONOTHERAPY AS AN ASSISTANT IN THE TREATMENT OF MASTITIS, IN LACTATING COWS<sup>1</sup>

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**ABSTRACT:** The action of Ozone (O<sub>3</sub>) as a germicide, against different pathogens has been studied; and has revealed the biological effects with therapeutic benefits [1-5]. Ozone inhibits growth and causes the death of gram negative and gram-positive bacteria, exposed to the ozone concentration of 0.167/ µg /min/L, at different times, produced ultra-structural changes in the bacteria, showing deformation and sudden damage with surface destruction, collapse and cell lysis [6]. The present work was developed in the “El Salado” zootechnical

<sup>1</sup> This article was published in 2019 by Juniper Publishers. (<https://juniperpublishers.com/jdvs/pdf/JDVS.MS.ID.555876.pdf>). Some authors have been eliminated from the original paper due to political issues.

post, belonging to the Faculty of Veterinary Medicine and Zootecnics of the “Benemérita Universidad Autónoma de Puebla”, in the production module of Milk Bovines, which has cows of the Holstein- Frisean and Jersey breeds, under a system of intensive handling, being milked twice a day (every 12 hours), in a 4-box fishbone milking parlor and with a Westfalia brand milking equipment; where, after identification of subclinical mastitis, by performing the California Mastitis Test on each cow, the readings were made and three experimental series were applied, using different concentrations of the ozone-oxygen mixture, in the affected mammary quarters of nine cows in production. After 4 days of treatment with the ozone / oxygen mixture, a 50% improvement was observed in the affected rooms, reflected in a reduction of somatic cells and leukocytes per milliliter of milk; being the dose that obtained better results, the one of 11µg of ozone in a mixture of ozone- oxygen in 300ml of volume, that were instilled in the affected breast rooms. It is concluded that the effect of intramammary administration of the ozone / oxygen mixture (MOO) is presented as an adjuvant in the innovative treatment of subclinical and clinical bovine mastitis, representing a real alternative, being necessary to establish effective doses and volumes for said end; Therefore, the indiscriminate use of antimicrobials would be avoided, establishing this ozone therapy as an effective, safe, cost-effective method, with zero days of withdrawal and without the risk of having drug residues in milk.

**KEYWORDS:** Ozone therapy. Subclinical mastitis. Milk quality.

## 1 INTRODUCTION

Mastitis is a serious disease suffered by milk-producing animals, causing large economic losses, due to the reduction in milk production, as well as its low nutritional value. Mastitis is characterized by the occurrence of physical, chemical and bacteriological changes in milk including pathological abnormalities of the mammary gland tissue. Mastitis affects the quality of milk in terms of its composition and production. The extent of the various changes in milk composition depends on the inflammatory response [7]. Somatic cells are the main epithelial cells that have detached from the glandular lining and white cells (leukocytes) that penetrate the mammary gland in response to a specific damage or infection. Somatic milk cells include 75% of leukocytes, such as neutrophils, macrophages, lymphocytes and 25% of epithelial cells. During inflammation (mastitis) the greatest increase in SC is due to the influx of neutrophils into the milk to fight the infection and has an estimated 90% [8,9]. The most precise relationship between intramammary infection and somatic cell count (SCC) can be assessed at the quarter level when the SCC exceeds 200,000 cells / ml [10]. In addition, the CCS in healthy rooms is consistently low and usually below 200,000 cells / ml [11]. In subclinical mastitis, the pathogens do not cause sufficient destruction of the alveolar tissue, so that it is reflected in the characteristics of the milk, but the SCC is increased indicating the infection of the milk [12].

In clinical mastitis, clinical signs can be recorded as weak, moderate and severe [13]. *Staphylococcus aureus* is considered one of the most virulent bacterial species that cause bovine mastitis (MSC) and clinical mastitis (MC) [14]. The administration of intramammary antibiotics is the most common method used for the treatment of bovine mastitis. However, at present this treatment is having few results regarding the recovery of the infection, mainly due to the bad management and the development of resistance by pathogens. Such is the case of the identification and characterization of methicillin-negative coagulase *Staphylococcus*-resistant in bovine mastitis [15,16]. The treatment of acute bovine mastitis during the period of breastfeeding represents one of the greatest economic losses of livestock production. This is related to the cost of treatments (veterinary service and drugs), the decrease in production and the sale price of milk, as well as the increase in somatic cell count in the milk sample volume, the increase in the number of cows discarded and the increase in losses due to the presence of antibiotic residues in milk and meat [17-19].

A range of production systems coexist in Mexico, ranging from the most traditional, in the hands of farmers in isolated regions of the country, to large modern companies, vertically and horizontally integrated. The technology required by the various systems is very different and MVZs must be very aware of the scope and limitations of each method or input they apply or recommend. The monitoring of the quality of products has a wide social demand and also plays a strategic role in the protection of our internal market, access to markets abroad and in making our country more attractive for these important economic activities. Therefore, this problem must be addressed by trained professionals, who can provide effective alternatives, easy to apply and with affordable costs, which guarantee the quality of food of animal origin. The presence of mastitis is a very serious problem for dairy farming and is one of the main causes of economic losses for the farmer and / or producer. In the United States, annual losses are estimated at around \$ 2,000,000. ([20], University of Oviedo, Gijón, Asturias). World losses, annual due to mastitis, have been estimated at US \$ 35 billion [21,22]. The greatest losses result from the reduction in milk production due to subclinical mastitis. In other studies, conducted in herds located in the highlands of Mexico, the prevalence of subclinical mastitis has been estimated at 20.80% in Tizayuca, Hidalgo, and 81.10% in stables around the Federal District [23-31].

Hygiene in cowsheds and milking place are very important and everyone knows that the lower the level of hygiene, the greater the risk of infection. Dr. Carlos Concha Bascuñan, M.V.Z. specialist of the University of Chile, recommends the application of drying knobs or treatments only in the case of cows with positive results to bacteriological

cultures carried out with the secretion existing during the period, has found that more than half of the cows, in The dry period is bacteriologically negative and all they need is a good external nipple sealer. By routinely applying antibiotics to all cows when they are dried, even in completely healthy udders, we soon create resistance, which will make it difficult to treat clinical cases. The Nordic countries, with a very careful policy “prohibition of the prophylactic use of antibiotics” as a treatment for cows on drying without having carried out bacteriological tests, has allowed them to present only 10 to 20% resistance to penicillins, versus 40 60% of the rest of Europe [32-39]. Due to the above, the use of ozone as a treatment in subclinical mastitis, will allow us to have zero days of withdrawal in milk, perfectly sterilize the room of bacteria, viruses and fungi, due to its potent oxidizing action, it will not allow the resistance effect of microorganisms and We will have the possibility to enjoy a milk free of antibiotics, anti-inflammatory, with moderate somatic cells and with a higher protein quality, both milk and derivatives.

## 2 MATERIAL AND METHODS

The realization of this work was carried out in the “El Salado” zootechnical post, belonging to the Faculty of Veterinary Medicine and Zootechnics of the Benemérita Universidad Autónoma de Puebla, which has cows of the Holstein-Frisean and Jersey breeds, under an intensive management system, being milked twice a day (every 12 hours), in a 4-box fishbone milking place.

### 2.1 INCLUSION CRITERIA:

- Cows with clinical and bacteriological diagnosis of clinical mastitis.
- Cows diagnosed with subclinical mastitis.

### 2.2 EXCLUSION CRITERIA:

- Cows that are under antibiotic or other therapeutic treatment.
- Cows that show signs of worsening of the clinical picture during treatment with the MOO.

### 2.3 EXPERIMENTAL SERIES GROUPS:

The animals were divided into experimental series of 3 cows in each group.

Experimental series 1: Dose of 11mg of ozone in 300ml of volume.

Experimental series 2: Dose of 35mg of ozone in 300ml of volume.

Experimental series 3: Dose of 50mg of ozone in 300ml of volume.

## 2.4 JUSTIFICATION FOR THE SELECTION OF MOO CONCENTRATIONS FOR EACH EXPERIMENTAL SERIES:

The treatments of the MOO by intramammary route will be carried out every 24 hour after discharging the milk from the room and after disinfecting the room with an antiseptic solution.

## 2.5 STUDY SUBSTANCES AND / OR REFERENCE:

The MOO, obtained from an Ozone Generator for Medical Use, Model MEDIC-O3 PORTATIL, Manufactured by OZONO CARBAR ´S. Serial No. BRJ17-960. The measurement was carried out by means of an ozone analyzer model MINI-HICON BENCH with serial number 170107 with a capacity of up to 400 g / Nm<sup>3</sup> with temperature and pressure compensator, of American origin.

Equipment Accuracy + - 2 µg / ml.

Maximum Standard Deviation: 0.11%

## 2.6 METHOD OF ADMINISTRATION AND / OR EXPOSURE AND REASON FOR BEING EMPLOYED:

Intra-mammary route: After disinfection of the nipple area of the room, with an iodine-based disinfectant solution, an intra- mammary cannula is placed, which is connected with the MOO- loaded syringe, it is slowly discharged until the volume of 300 ml is completed determined for the application. It will be applied every 24 hours for 4 consecutive days.

## 2.7 MEASUREMENTS TO BE PERFORMED:

- a. Review of the medical history of each cow registered in the medical control cards of the production module.
- b. Identification of race, age, days of milk production.
- c. Registration of physiological constants.
- d. Carrying out the mastitis California test.
- e. Performing the somatic cell count test.
- f. Identification of the rooms affected by clinical mastitis and their antimicrobial treatment.
- g. Observation of the response to ozone treatment or recurrence.

## 2.8 SOMATIC CELL COUNT (SCC)

Milk samples were taken from the affected rooms and SCC counting was performed using the Porta Check SCC method; taking as a reference, the count greater than 200,000 cells / ml and apparently without morphological changes in milk, will be classified as subclinical mastitis (SCM). While a SCC from 5,000,000 cells/ml, accompanied by morphological changes of milk such as lumps and color changes, it will be classified as clinical mastitis (CM). This diagnostic method will be performed before and after the end of the treatment cycle with the substance under test (Table 1).

Table 1: This diagnostic method will be performed before and after the end of the treatment cycle.

		07-11-18						10-11-18							
		California Mastitis Test				Ozone Dose		Type of Mastitis		Type of Mastitis California Mastitis Test				Ozone Dose	
No. Cow	c) Clínica	AI	AD	PI	PD		No. Cow		AI	AD	PI	PD			
	s) ubclínical														
4	s)	-	2	1	-	11µg/300ml	4	s)	-	-	T	-	11µg/300ml		
69	s)	-	T	2	2	11µg/300ml	69	s)	-	-	1	1	11µg/300ml		
49	s)	2	1	2	2	11µg/300ml	49	s)	2	1	T	1	11µg/300ml		
28	s)	-	3	2	-	35µg/300ml	28	s)	2	2	2	T	35µg/300ml		
160	s)	-	3	3	2	35µg/300ml	160	s)	-	-	-	-	35µg/300ml		
38	c)	C	-	-	-	35µg/300ml	38	c)	2	-	-	-	35µg/300ml		
19	s)	-	-	3	-	50µg/300ml	19	s)	-	-	2	3	50µg/300ml		
77	s)	2	-	2	-	50µg/300ml	77	s)	1	-	1	-	50µg/300ml		
99	s)	2	-	-	-	50µg/300ml	99	s)	2	-	-	-	50µg/300ml		

## 3 RESULTS

After 4 days of treatment with the ozone / oxygen mixture, a 50% improvement is shown in the affected fourth.

The cow No. 38 that presented clinical mastitis in the fourth AI, after treatment improved, showing in the California mastitis test a clear positive reading 2, with reduction in the number of leukocytes and somatic cells in said room.

It can be seen that the most effective dose of the ozone / oxygen mixture is 11 to 35µg / ml, in a volume of 300ml (Table 2).

Table 2: Milk sample from thermo tank.

No. of Cow	The Affected Fourth	SCC at the Beginning	SCC the End
4	AD	1,720,000/ml	630,000/ml
38	AI	>3,000,000/ml	950,000/ml

24	PD	873,000/ml	230,000/ml
Milk sample from thermo tank			
SCC at he beginning		SCC the end	
281,000/ml		253,000/ml	

## 4 DISCUSSION

Taking into account the high incidence of mastitis in the dairy farming industry, as well as the economic losses due to said disease, it is appropriate to carry out its diagnosis in the field and treat in the best way and in each case in particular, being a good option, the administration of correct doses of the ozone-oxygen mixture. Several works show that the Ozone / Oxygen Blend has a high germicidal power and in the present work, an effectiveness of the MOO was obtained as a treatment for bovine mastitis. Antibiotic treatments are not entirely effective because of the high incidence of resistance developed by the microorganisms that cause bovine mastitis. The use of ozone in gas has no withdrawal period or leaves metabolites in milk. Therefore, it is rational to study and determine the optimal MOO concentrations for the treatment of clinical and subclinical mastitis in cattle, according to the causative germs.

## 5 CONCLUSION

The effect of intramammary administration of the ozone / oxygen mixture (MOO) as an innovative treatment of subclinical and clinical bovine mastitis is investigated, establishing effective doses for this purpose. Avoid the indiscriminate use of antibiotics, establish an effective, safe, profitable method, with zero days of withdrawal and without risk of having residues of medicines in the milk. The sterilization of treated breast rooms is checked by physical and biological methods. A practical, viable and low-cost application protocol is established to control the presence of clinical mastitis in the dairy herd.

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## DIAGNOSTICS IN A PUG DOG WITH ALLERGY REACTION ON RABIES VACCINE, CLINICAL PICTURE AND ATOPIC DERMATITIS– CASE REPORT

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**ABSTRACT:** The presentation of this case is a dog of the age of 7 years. The clinical picture of this dog was monitored periodically for years on an outpatient basis before a definitive diagnosis of Atopic Dermatitis was made. The symptoms included ear reactions with mixed infections and swelling, increased secretion from the eyes and nose with occasional changes in the skin. Diarrhoea was rarely present. After the annual vaccine, the dog showed a rapid allergic reaction after four years of regular rabies supplementary vaccines. The same reaction was after two years on the rabies vaccine again. During the allergy testing, other diagnostics were also performed according to usual procedures. In the haematology findings of the complete blood count, changes were recorded in white blood cell parameters: lymphopenia, neutropenia, eosinopenia and monocytosis. A positive reaction to both types of allergens was confirmed by serological testing using the Elisa method of dog serum for inhalant

allergens and food allergens. A positive and highly positive result was obtained for even 20% of inhalant allergens and 37.71% of food allergens. A positive reaction to an external (OUT) allergen group of 15% included pollen while 5% of positive reactions included indoor (IN) allergen group. The total number of tested allergens is 40+21 on a large panel. Screening Elisa method is recommended for easier and faster determination of therapy in patients with Allergic Dermatitis (AD).

**KEYWORDS:** Atopic Dermatitis. Dog. Food allergens. Inhalant allergens. SAT.

### 1 INTRODUCTION

Atopic dermatitis (AD) is a genetically predisposed skin disease, related to IgE-mediated hypersensitivity. The serum allergen-specific IgE test (SAT) is used to detect allergen-specific IgE antibodies. This type of test identifies inhaler allergens from the external (OUT) and internal (IN) environment (Tommaso et al 2021), and food allergens. Serum is used as a test sample. According to the conducted clinical studies, this type of allergy testing is indicated in the case of a visible clinical picture or after monitoring the patient at the veterinarian for a long period (Hensel et al 2015, Tommaso et al

2021). Depending on the type of allergen, which can be seasonal, external, internal or food allergen, clinical signs can be manifested at different times of the year. The external (OUT) group of inhalant allergens most often produces clinical signs in pets when pollen is present in the air during the flowering period. Groups of internal (IN) inhalant allergens (dust mites) cause symptoms in pets during the most intensive period of stay in the house (Chermprapai and Thengchaisri 2021) during rainy days, in winter, in a subtropical climate. From other groups of allergens such as those from food - symptoms can appear at any time of the year (Hensel et al 2015). The clinical picture of such pets without adequate therapeutic control and a medicated diet can be very frustrating for the owner until a definitive diagnosis is made. Such examinations are expensive and require patience in monitoring the patient as well as trust with financial support from the owner.

## 2 MATERIAL AND METHODS

### 2.1 ANAMNESTIC AND CLINICAL FINDINGS

The presentation of this case in a dog of the age of 7 years includes a clinical picture with applied diagnostics. The clinical picture of a pug dog was monitored periodically for 4 years on an outpatient basis before a definitive diagnosis of Atopic Dermatitis was made. The symptoms included ear reactions (otitis externa) with mixed infections and swelling, increased secretion from the eyes and nose with occasional changes on the skin. Diarrhoea was rarely present. According to the applied regular annual vaccine Nobivac Rabies (MSD) dose of 1 ml, the dog showed a rapid allergic reaction after four years of regular booster vaccines. The same reaction was on the vaccine Rabigen mono (Virbac) dose of 1 ml. Tests and examinations of the complete blood count, and biochemical parameters were performed after the first allergic reaction. In the findings of the complete blood count, changes were recorded in most white blood cell parameters manifested as lymphopenia, neutropenia, and eosinopenia, while monocytes were seven times more elevated compared to reference values. A positive reaction was confirmed by serological testing using the Elisa method of the dog's serum to inhalant allergens and food allergens.

### 2.2 BLOOD SAMPLING

Venous blood was taken from the jugular vein or other accessible veins by venipuncture. Test tubes with anticoagulant EDTA were used for complete blood count testing, while test tubes with a separator were used for biochemistry sampling. For a complete blood count, 0.5-1 ml of blood was sampled. To obtain serum from test tubes

with a separator for biochemistry 3-4 ml of blood was sampled. To separate the serum, a centrifuge is used at 3000 revolutions in a time interval of 10-15 minutes.

### 2.3 COMPLETE BLOOD COUNT ANALYSIS

Examined parameters in the complete blood count after sampling: total leukocyte (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, LUC, percentage of neutrophils, percentage of lymphocytes, percentage of monocytes, percentage of basophils, erythrocytes (RBC), mean corpuscular haemoglobin (MCH), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), haemoglobin, platelets (PLT), mean platelet volume (MPV) and platelet crit (PCT). The tests were carried out on the Advia 120 device, Siemens in the Vetlab laboratory, in Belgrade.

### 2.4 COMPLETE BIOCHEMICAL TESTS

Tested parameters of biochemistry: total proteins, globulins, albumins, A/G, total bilirubin, cholesterol, glucose, urea, creatinine, U/C, ALP, alpha-amylase. Tests were performed on the AU480, Beckman Coulter in the Vetlab laboratory, Belgrade.

### 2.5 DETECTION OF ANTIBODIES IN THE SERUM OF TESTED DOG

Standardised Elisa method testing with manufacture prescription (Invitros Laboratorios, Madrid, Spain) was used to detect a positive test for the investigated allergens in dog sera. The method is standardised in Spain. The large panel consists of 40 sites for testing inhalant allergens and 21 sites for testing allergens of food origin. Wells (eng. wells) possess specific antigenic proteins, which in the case of the presence of specific antibodies of the IgE class lead to a positive test reaction. The test result is semi-quantitative, and depending on the intensity of the reaction, the obtained values are classified as negative, borderline, positive and highly positive. Before reading the reaction, three well solutions are used and washed at different time intervals. After the last washing of the basin, the reaction is read 18 hours after the end of incubation. Absorbance reading is performed on a spectrophotometer (Thunderbolt, Gold Standard Diagnostics, USA) at two wavelengths, 405 nm and 650 nm. The classification of reaction intensity is determined based on the reading value of the calibrator of known concentration within each individual reading. The total number of allergen tests is 40+21 in a large panel. The panel of inhalant allergens includes 40. The panel of food allergens includes a total of 21. The test was performed in the accredited veterinary laboratory for clinical diagnostics Vetlab in Belgrade, Serbia.

### Tested inhaler allergens

OUT serum allergen-specific IgE tests: *Phleum pretense*, *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Cynodon dactylon*, Cereals mixture, *Rumex crispus*, *Taraxacum vulgare*, *Artemisia vulgaris*, *Plantago lanceolata* *Parietaria judaica*, *Chenopodium album*, *Urtica dioica*, *Ambrosia elatior*, *Brassica* spp., *Platanus hybrida*, *Olea europaea*, *Betula alba*, *Corylus avellana*, *Alnus glutinosa*, *Lingustrum vulgare*, *Pinus* spp., *Populus alba*, *Quercus rubor*, *Ulmus campestris*, *Salix* spp and *Cupressus arizonica*.

IN serum allergen-specific IgE tests: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Alternaria alternata*, *Aspergillus niger*, *Penicillium notatum*, *Mucor* spp, *Cladosporium herbarum*, *Malassezia*, *Staphylococcus*, and fleas.

### Tested food allergens

Serum allergen-specific IgE tests for food: beef, turkey, chicken, pork, lamb, duckling, rabbit, venison, mixed fish, egg, cow's milk, wheat, oats, rice, soy, corn, beetroot, carrots, potatoes, peanuts, yeast.

## 3 RESULTS

### 3.1 CLINICAL FINDINGS

Pictures of earlier clinical findings in a dog with ear changes (Picture 1) and on the skin coat (Picture 2). The appearance of a dog with an allergic reaction after the administration of a regular vaccine with generalized tissue swelling on the dog's face (Picture 3 and Picture 4).

Picture 1. Otitis externa on the pug dog.



Picture 2. The skin on the pug dog.



Picture 3. Pug dog face after the booster vaccine.



Picture 4: Generalized tissue swelling on the dog's face.



### 3.2 COMPLETE BLOOD COUNT ANALYSIS AND BIOCHEMICAL TESTS

The ratio of reference and obtained values after the analysis of complete blood count (Figure 1). In the haematology findings of the complete blood count, changes were recorded in white blood cell parameters: lymphopenia, neutropenia, eosinopenia and while mocytes. The ratio of reference and obtained values after the analysis of the complete biochemical test is shown in Figure 2.



Figure 1. Ratio haematology reference values and analyze values in dog.

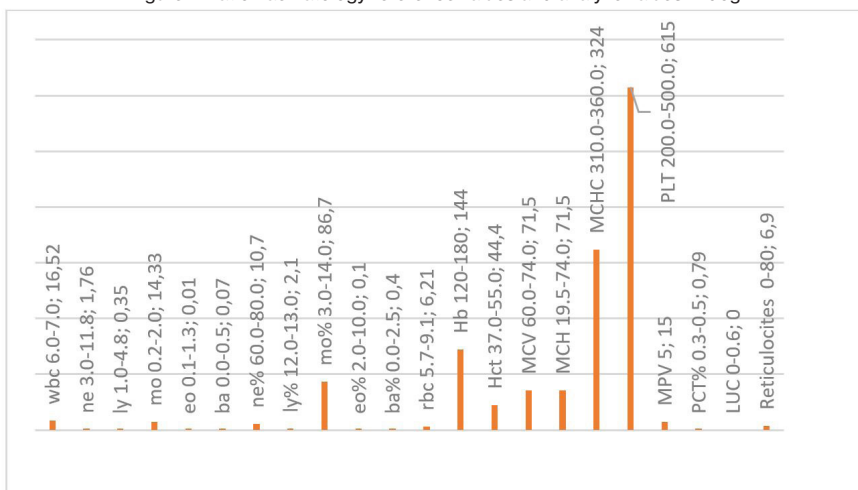
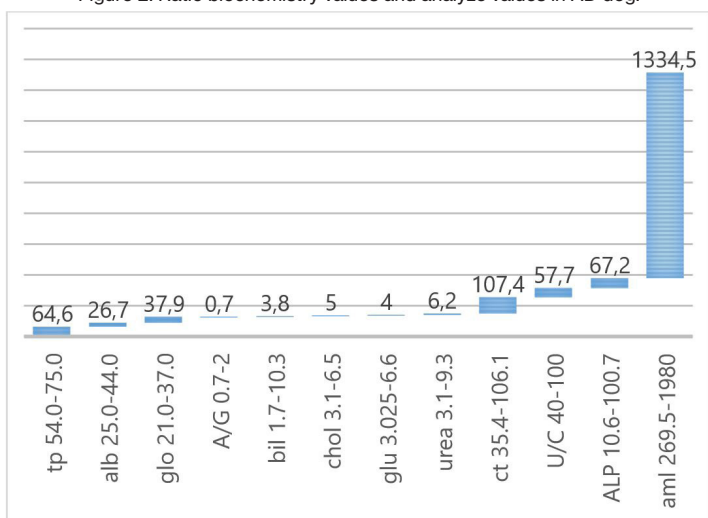


Figure 2. Ratio biochemistry values and analyze values in AD dog.



**Figure 1, Legend:** wbc-total leukocyte, ne-neutrophils, ly-lymphocytes, mo-monocytes, eo-eosinophils, ba-basophils, ne%-percent neutrophils, ly%-percent lymphocytes, mo%-percentage monocytes, ba%-percentage basophils, rbc-erythrocytes, LUC, MCH-mean corpuscular haemoglobin, Hct-hematocrit, MCV-mean corpuscular volume, MCHC-mean corpuscular haemoglobin concentration, Hb-haemoglobin, reticulocytes, PLT-platelets, MPV-mean platelet volume and PCT-platelet crit.

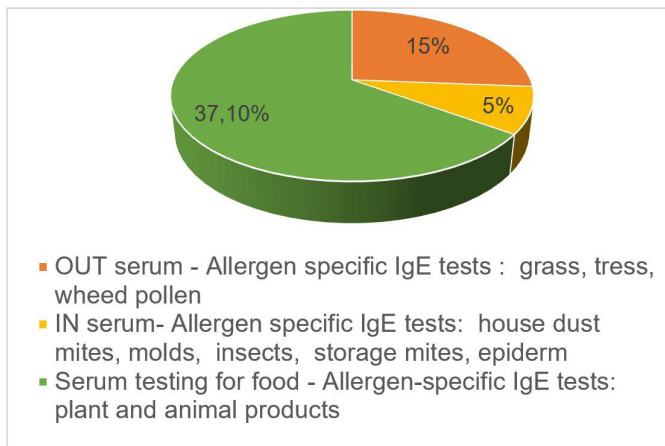
**Figure 2, Legend:** tp-total proteins, glo-globulins, alb-albumins, glu-glucose, A/G-albumin globulin ratio, bil-total bilirubin, chol-cholesterol, urea, ct-creatinine, U/C- ratio urea creatinine, aml-alpha amylase, ALP.

### 3.3 DETECTION OF ANTIBODIES IN THE SERUM OF TESTED DOG

The aforementioned tests for the presence of specific antibodies of the IgE class included in the large panel represent inhalant allergens and allergens originating from food

in the said dog (Figure 3). The dog showed a positive reaction to allergens from the pollen group *Quercus robur*, *Phleum pretense*, *Dactylis glomerata*, *Poa pratensis*, *Lolium perenne*, *Cynodron dactylum*, while within the group of indoor allergens *Dermatophagoides farina*, *Dermatophagoides pteronyssinus*. The dog showed positivity in the following investigated food allergens: rice, oats, corn, wheat, fish and mixed fish, beef, turkey, and lamb.

Figure 3. Serum allergen-specific IgE tests (SAT) and percentage of positive reactions in pug dog with AD.



#### 4 DISCUSSION

Positive findings on allergens in the tested pug dog were obtained after the serum test was performed during the existence of the clinical picture. Positive findings in dogs in preliminary studies in Italy (Tomasso et al 2021) also gave positive reactions during the examination performed during the existence of the clinical picture. The diagnosis is based on the anamnesis, the clinical picture and according to some authors, also on the presence of itching (Marsella, De Benedetto 2017). In the dog that is being described, itching as a clinical symptom was not significant. Comparisons with other studies are made difficult by the circumstances of pollen distribution in different geographical areas, climate changes, plant characteristics, seasons (Tomasso et al 2021). The positive inhalatory allergens of the tested dog belong to the group of external (OUT) allergens, mostly grass pollen and one tree pollen. When it comes to pollen reactions to grasses, and especially the grass *Cynodron dactylum*, it can be said that a positive reaction in AD dogs was recorded in up to 60%, for example, in Italy (Tomasso et al 2021), while in the examined dog it was measured as highly positive. In some studies, they report that allergic reactions in dogs to inhalant internal allergens are much more common (Chermpapai and Thengchaisri 2020). In a study in Norwegian dogs, it was determined that an allergic

reaction to the mites *D. farinae* and/or *D. pteronyssinus* (Bjelland et al 2014) was present in a higher percentage. A positive reaction was obtained in the results of the tested dog to the mentioned mites. Trials in Europe and the USA have produced similar results in some studies. Practical guidelines for diagnosis exclude other diseases with an applied diagnosis of allergen-specific immunoglobulin E in serology (ASIS). Food reactions can be caused in two ways non-immune mediated (food intolerance) and immune-mediated which includes IgE-mediated hypersensitivity (food allergy) (Hillier, Griffin 2001). Reactions to food components can be clinically presented as Atopic Dermatitis (AD) and common gastrointestinal signs are diarrhoea, vomiting, soft stools, flatulence (Picco et al 2008, Favrot C et al 2010, Hensel et al 2015). Vaccination regimens, parasitic diseases, and glucocorticosteroids are some of the factors that can affect the level of IgE in the serum (DeBoer, Hillier 2001). Vaccination in the examined dog was reflected in a strong sensitisation of the organism. Such tests are effective because they do not require sedation, reduce the risk of anaphylactic shock, and do not immediately affect the therapy given to the patient (Chermprapai and Thengchaisri 2020).

## 5 CONCLUSION

Screening Elisa method is recommended for easier and faster determination of therapy in patients with Allergic Dermatitis (AD).

## 6 ACKNOWLEDGMENT

There is no conflict of interest.

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# CAPÍTULO 14

## PARASITIC CONTAMINATION OF PUBLIC PLACES IN BELGRADE AND ITS CONTROL RESULTS OF A THIRTY-YEAR STUDY (1993-2023)

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concurrently posing public health risks due to increasing populations of both owned and stray dogs. Urban areas globally are grappling with serious challenges, including injuries from dog attacks and contamination from dog feces, which can harbor zoonotic parasites. This study examines the contamination levels in public spaces, particularly in Belgrade, where a comprehensive monitoring program has been implemented since 1993. The research tracks the prevalence of harmful parasites in parks and playgrounds, revealing alarming contamination rates that pose significant health risks. Despite substantial improvements in urban hygiene due to public health initiatives and responsible pet ownership, challenges remain. The study outlines effective management strategies, including regular parasitological monitoring, public education, and legislative enforcement. Findings suggest that humane approaches to managing stray dog populations, alongside continuous community engagement, can significantly reduce contamination levels and improve public health outcomes.

**KEYWORDS:** Urban health. Stray dogs. Zoonotic parasites. Public hygiene. Animal welfare. Contamination control. Belgrade. Epidemiology. Public education. Legislative enforcement.

## 1 INTRODUCTION

Pets, especially dogs, play a significant role in the lives of modern people, especially in

**ABSTRACT:** The presence of pets, particularly dogs, significantly enriches urban life by offering psychological benefits and enhancing socialization among children, while

the urban environment. Alienation, stress, and other factors that burden the city man are often psychologically overcome by acquiring a dog, which, with its devotion, represents excellent psychotherapy (which in some countries is generally accepted as a prevention of psychological and stress-related diseases such as high blood pressure, heart disease or increased cholesterol). It has also been noted that socialization develops more easily in children who have pets, and in old people, life expectancy increases and the tendency to depression decreases

Unfortunately, in addition to this favorable influence, the presence of dogs in urban areas has another side. The increase in the number of owned and abandoned dogs in cities is a current problem of global proportions. Every year, the number of people injured by dogs increases, and their excrement is a particular problem. In the close cohabitation of stray dogs and pets that are in the immediate environment of people, there is constant contamination of public areas, especially streets and parks, with dog feces. In the urban environment, green areas and parks are the main places where children play and they are resting places for city people, but they are also places where dog owners take their pets out and places where we meet non-owner dogs - usually former pets. If it is known that a dog excretes an average of 100 grams of feces per day, it is easy to determine, based on an insight into the number of animals in the city, that the amount of dog excrement that is excreted daily passes several tons per day.

Dog excrement, apart from its unpleasant appearance and smell, represents an epidemiological danger considering that dogs are carriers and real hosts of a large number of species of zoonotic parasites. Among the many types of parasites that dogs are real hosts for *Toxocara canis*, *Ancylostomidae* sp., *Echinococcus granulosus*, *Dipylidium caninum*, *Taenia* sp., *Trichuris vulpis* and *Strongyloides stercoralis* and oocysts of the protozoa *Giardia intestinalis*, *Amoeba* sp. and *Cryptosporidium* sp.

Dog owners are certainly the population most exposed and threatened by parasitic zoonoses. Toxocariasis has been observed much more often in dog breeders who do not take enough care to deworm their dogs, especially since toxocariasis is incomparably more common in puppies than in adult dogs. Refers to children who tend not to wash their hands after contact with animals or have a habit of geophagy. In semi-rural and rural areas, dogs defecate unhindered on vegetables, so unwashed vegetable food can be a significant source of infection if it is not well washed. Likewise, in urban areas, feces that are on in the streets during the summer it is dried up and parasite eggs can infect people through inhalation. In the case of rain, the excrement dissolves, creating a microemulsion on the pavement, lawns and public areas, and

together with parasite eggs, they are easily carried into houses on shoes, becoming a source of infection.

## 2 CONTAMINATION OF URBAN AREAS IN THE WORLD

Contamination of urban areas with dog excrement is a ubiquitous problem of global proportions to which highly developed countries are equally exposed, almost to the same extent as third world countries. The difference is only in the types of parasites (due to geographical position, climatic and socioeconomic factors) and the dogs that carry them. In countries with a high standard, they are pet animals, while developing countries are threatened by stray dogs. Either way, *Toxocara canis*, *Ancylostomidae* spp., *Echinococcus granulosus*, *Strongyloideus stercoralis*, *Dipylidium caninum*, *Giardia intestinalis* or *Amoeba* spp. they don't know borders, dog breeds or owner statutes, they will infect them all equally if given the chance.

In semi-rural and rural areas, dogs defecate unhindered on vegetables, so unwashed vegetable food can be a significant source of infection if it is not well washed. Likewise, in urban areas, feces that are on in the streets during the summer it is dried up and parasite eggs can infect people through inhalation. In the case of rain, excrement dissolves, creating a microemulsion on pavements, lawns and public areas, and together with parasite eggs, they are easily brought into houses on shoes, becoming a source of infection. Looking back at the results of parasitological surveys of public areas in cities around the world, we will see that this contamination with eggs of zoonotic parasites constitutes a global problem.

For example, in Madrid it was 9%, London 15-17%, Michigan 19%, Kansas 20.6%, Utrecht 23%, Belgrade, Paris and Prague 28%, Dublin 32%, Naples 48%, Tokoshima 63% etc. If it is known that more than 5% of polluted surfaces represent a serious danger to people's health, we are of the opinion that this should not be commented on.

The ecological and epidemiological implications of the problem are attracting the attention of eminent experts, and although this problem on a global scale is not new, its solution is still the subject of numerous analyses, discussions and conflicting opinions, ranging from the principle of total removal of animals from the streets to much more humane and acceptable solutions such as NKS ( No Kill Strategy) and CNR (Catch-Neuter-Release). Such programs are also supported by leading international animal protection organizations WSPA (The World Society for the Protection of Animals), RSPCA (Royal Society for the Prevention of Cruelty to Animals International), IFAW (International Fund for Animal Welfare), HSI (Humane Society International ), the World Small Animal

Veterinary Association and the Alliance for Rabies Control, so the creation of such a strategy requires full cooperation with them and with all other organizations whose goal is animal welfare.

Public interest in the welfare of animals is growing and is increasingly present in the world, as well as in our society. This trend inspires numerous debates and discussions about animal welfare as well as ways and problems that arise when solving the problem of abandoned animals. Factual and scientific knowledge in this area must support and inform ethical decisions, and especially the need to understand the affective states of animals (the problem of interaction with people) and their needs in relation to the environment in which they move and find themselves. Animal welfare is the course that we should follow in applying methods to solve the problem of abandoned animals.

The positions to be defended, which concern the welfare of animals, require scientific knowledge about the biology of animals, in order to enable the closest determination of their physiological, health and psychological needs, as well as to better solve the problems and issues of their impact on the environment and people's health.

### 3 EPIDEMIOLOGICAL IMPORTANCE OF THE MOST COMMON HELMINATES OF DOGS

The most common and perhaps the best known parasitic diseases transmitted by dogs are echinococcosis, toxocarasis and hookworm. None of them develop into adult parasites in humans, but their developmental forms (cysts or migratory larvae) cause serious illnesses, even death.

#### 3.1 ECHINOCCOCOSIS

Echinococcosis is a zoonotic disease that is caused by tapeworms of the genus *Echinococcus*. *Echinococcus* adult worms develop from protoscolices and are typically 6 mm or less in length and have a scolex, neck and typically three proglottids, one of which is immature, another of which is mature and the third of which is gravid (or containing eggs). Carnivores act as definitive hosts for the parasite, and harbour the mature tapeworm in their intestine. The definitive hosts are infected through the consumption of viscera of intermediate hosts that contain the parasite larvae. A number of herbivorous and omnivorous animals act as intermediate hosts of *Echinococcus*. They become infected by ingesting the parasite eggs in contaminated food and water, and the parasite then develops into larval stages in the viscera.



Humans act as so-called accidental intermediate hosts in the sense that they acquire infection in the same way as other intermediate hosts, but are not involved in transmitting the infection to the definitive host. The two most important forms, which are of medical and public health relevance in humans, are cystic echinococcosis (CE) and alveolar echinococcosis (AE).

Human infection with *E. granulosus* leads to the development of one or more hydatid cysts located most often in the liver and lungs, and less frequently in the bones, kidneys, spleen, muscles and central nervous system. The asymptomatic incubation period of the disease can last many years until hydatid cysts grow to an extent that triggers clinical signs, however approximately half of all patients that receive medical treatment for infection do so within a few years of their initial infection with the parasite.

Alveolar echinococcosis is characterized by an asymptomatic incubation period of 5–15 years and the slow development of a primary tumour-like lesion which is usually located in the liver. Clinical signs include weight loss, abdominal pain, general malaise and signs of hepatic failure.

### 3.2 TOXOCARIASIS

The large roundworms (ascaridoid nematodes) of dogs and cats are common, especially in puppies and kittens. Two species, *T. canis* and *T. leonina*, infect dogs and are found globally. For all species, adult parasites are stout cream-colored nematodes that are found in the small intestine and typically range from 3 to 15 cm in length. In dogs, the most important roundworm is *T. canis*, not only because its larvae may infect humans but also because infections are generally common and may impact puppy health. Also, fatal infections may occasionally occur in young pups. In puppies, the most common mode of infection is transplacental transfer. If pups < 3 months old ingest embryonated infective eggs, the hatched larvae penetrate the intestinal mucosa and reach the lungs via the liver and bloodstream. Then they are coughed up and swallowed, maturing to egg-producing adults in the small intestine. The prepatent period ranges from 2 to 3 weeks after prenatal infection to 4 to 5 weeks after ingestion of eggs or paratenic hosts.

Dogs typically develop immunity to *T. canis* with age. However, during the perinatal period, the immunity of the bitch to infection is partially suppressed, and substantial numbers of eggs may be passed in the feces of the bitch. Development of these patent infections appears to be associated with maturation of arrested larvae in the bitch, which migrate to the intestine via the lungs. Patency may also occur as a result of ingestion and maturation of larvae that are passed in the feces of puppies. Eggs of *T. canis* become infective (larvae) after ~2–4 weeks in the environment.

Human infection with *T.canis* occurs through ingestion of parasite eggs. Larvae emerge from the ingested eggs in the intestines and start migrating through the bloodstream, and during migration they stop in the lungs, brain, heart, eyes and other organs, causing significant diseases (the disease is known as visceral larva migrans syndrome). Adult parasites do not develop in humans. A milder form manifests itself with skin changes and lymphadenopathy. The more severe form, which is most often encountered in children, is characterized by cough, chronic obstructive bronchitis, asthma, recurrent eosinophilic pneumonia, high temperature, enlargement of the spleen and liver, meningitis, encephalitis, epilepsy, convulsions, abdominal disorders, nausea, anorexia, myocarditis, myocardiopathies, etc. Fatal outcomes are possible.

*T leonina* is present in adolescent/adult dogs and is a less common infection than *T canis*. Dogs become infected via ingestion of infective eggs and infected paratenic hosts (eg, rodents). Infections are not zoonotic. As in dogs, *T leonina* infections are acquired via ingestion of infective eggs and larvae in paratenic hosts, eg, rodents; migration is restricted to the intestinal wall so that neither prenatal nor transmammary transmission occurs. Infections are not zoonotic.

### 3.3 ANCYLOSTOMA SP.

Hookworms (*Ancylostoma* spp, *Uncinaria stenocephala*) are common infections of dogs and cats, particularly puppies and kittens. Some species are zoonotic. Multiple hookworm species infect dogs and cats. *A caninum* is the principal cause of canine hookworm disease in most tropical and subtropical areas of the world and is considered the most pathogenic of the dog hookworms. *A caninum* has become more common than *U stenocephala* in dogs. Adult hookworms reside in the small intestine. *A caninum* males are ~12 mm long and females ~15 mm; the other species are somewhat smaller.

Thin-walled hookworm eggs in the early cleavage stages (2–8 cells) are first passed in the feces 15–20 days after infection; they complete embryonation and hatch in 24–72 hours on warm, moist soil. For all hookworm species, transmission may result from ingestion of infective larvae from the environment and additionally, in the case of *A caninum*, via the colostrum or milk of infected bitches. Infections with *A caninum* can also result from larval invasion via the skin; however, this route is of little significance for *U stenocephala*.

Skin penetration in young pups is followed by migration of the larvae via the blood to the lungs, where they are coughed up and swallowed to mature in the small intestine. However, in animals > 3 months old, *A caninum* larvae, after migration via the lungs, are

arrested in the somatic tissues, e.g., muscle, fat, and mucosa of small intestine. These arrested larvae may be activated during pregnancy, then accumulate in the mammary glands. Reactivation of dormant larvae, which may also occur after removal of adult worms from the intestine and for other unknown reasons, results in development of patent infections and is referred to as “larval leak.”

Human infections with members of the Ancylostomida spp. (*Ancylostoma caninum* and *Uncinaria stenocephala* in our environment) are caused by penetration of the larvae through the skin and ingestion. The migration routes are similar in both cases, but adult parasites never appear in humans. The disease manifests itself in the form of skin changes - redness, swelling, inflammation; often the surrounding lymph nodes can also be affected. More severe cases of dermatitis are also possible while fatal cases of this syndrome known as cutaneous larva migrans have not been reported.

Apart from them, *Strongyloides stercoralis*, *Dipylidium caninum*, *Taenia multiceps* and *Trichuris vulpis* are zoonotic helminths of dogs too.

#### 4 INFLUENCE OF ENVIRONMENTAL FACTORS ON THE PREVALENCE OF GEOHELMINATES

The vast majority of helminth eggs found in public areas are from the group of geohelminths - parasites whose development takes place in the soil, so that the thrown eggs become infectious for animals and humans only in the external environment. All over the world, the eggs of *T. canis* and *Ancylostomida* spp are the most numerous. - parasites with the greatest zoonotic potential.

The degree of infection of public spaces with geohelminths depends on the number of parasite eggs per kg of soil, and the speed of parasite development on the microclimate conditions prevailing in certain parts of the year. Thus, when studying this type of pollution and assessing the risk of human infections, we must be guided by the knowledge of bioclimatic conditions so that taking samples and interpreting the results are consistent with the actual situation on the ground.

The correlation between the climate (that is, the climatogram that shows it) and the appearance, distribution, number, and rapid development of living beings is very strong. If data on the vital optimum, development and generations of living beings during the year are included in the climatogram, it is then a bioclimatogram. Bioclimatograms are useful for showing, explaining or forecasting the occurrence or absence of a species in increased or decreased abundance. Therefore, there is a strong correlation of microclimate conditions with the dynamics of biological phenomena, and therefore a mutual correlation

of biological phenomena that take place in those conditions of the park areas of Belgrade were guided by a bio-climatogram according to Uvarovo in order to determine the optimal time of taking samples for inspection and evaluation of the obtained results.

Based on previous research, a change in parasitofauna was observed, which was caused by the influence of climate changes in Belgrade during the last decades. The prevalence of certain parasitic species is significantly lower, and at the same time, species of parasites that were not previously present in this area have appeared. This trend had its upward trend in the last few years, when there were more drastic climate changes - with mild winters, very hot summers, a large amount of atmospheric precipitation, etc. This was reflected in the biodiversity, prevalence and incidence of the parasitic fauna of the green areas of Belgrade in the period from 1993, when these continuous researches began until today.

Changes in the microclimate, which in the last few years had a significantly greater temperature oscillation (significant increase in average temperature and air humidity), are particularly favorable for the development of certain geohelminths. Looking from an epidemiological point of view, the dominant type of parasite is *T. canis* parasite, whose embryonic development in eggs takes place in the soil and which is directly dependent on the prevailing microclimate. At a temperature of 25 degrees, eggs embryonate in 7-12 days and at a temperature of 25 degrees in 5 days. Eggs are inactivated at a temperature higher than 37 degrees, before the formation of larvae. At a temperature of 50 degrees, they are inactivated in 5 hours, and at a temperature of 55 degrees in 1 hour. Direct sunlight inactivates them very quickly. in the specified period.

Changes in the microclimate, which in the last few years had a significantly greater temperature oscillation (significant increase in average temperature and air humidity), are favorable for the development of certain geohelminths, which have the greatest epidemiological significance as the cause of human toxocariasis, better known as larval migrans syndrome.

Based on previous research, a change in the parasitofauna was observed, which was caused by the influence of climate changes in Belgrade during the last decades. The prevalence of certain parasitic species is significantly lower, and at the same time, species of parasites that were not previously present in this area have appeared. This trend had its upward trend in the last couple of years, when there were more drastic climate changes - with mild winters, very hot summers, a large amount of atmospheric precipitation, etc. This was reflected in the biodiversity, prevalence and incidence of the parasitic fauna of the green areas of Belgrade in the period from 1993, when continuous research began until today 2023.

The conclusion of our research, which was carried out for thirty years, is that a change in the parasitofauna was observed, which was caused by the influence of climate changes in Belgrade during the last decades. The prevalence of certain parasitic species is significantly lower, and at the same time, species of parasites that were not previously present in this area have appeared. This trend had its upward trend in the last few years, when there were more drastic climate changes - with mild winters, very hot summers, a large amount of atmospheric precipitation, etc. With a significant decline in the hygiene of city parks and streets, at least as far as dog excrement is concerned, which irresponsible owners let defecate wherever they go and do not clean up after them, only worsen the epidemiological aspect of this problem.

## 5 METHOD OF PARASITIC CONTROL CONTAMINATION OF GREEN AREAS

In order to solve the problem of fecal and parasitological contamination of public areas basic starting point is a regular examination were green spaces and dog faces caught on them in order to gain insight into the epidemiological situation and start the rehabilitation and eradication of contaminated places. Also, it is necessary for local governments to define the program of non-proprietary control animals in their environment.

### 5.1 PLACES

Samples of soil and grass samples and dog faeces were taken from the parks and public places in central city municipalities - Stari Grad, Palilula, Vračar and Savski Venac. Examinations were carried out in the following parks and green area: Tašmajdan Park, Mali Tašmajdan, Čuburski Park, Manjež Park, Academic Park, Kalemegdan, Pionirski Park, Terazijski plato and Botanic Garden.

### 5.2 SAMPLING

Since 1993, every year, the same numbers of soil and grass samples and dog faeces were taken from the same parks (64 soil and 64 feces samples twice on year, each year). The material for examination is taken in the form of a group sample of soil, grass and dog excrement, the number of which depends on the size of the location, and sand from children's playgrounds is taken from each of the small pools in the mentioned locations. Each sample is packed in PVC bags, on the outside of which the location, sample number and date of sample collection are recorded.

Samples of grass and soil from green areas were collected from March to October. The material for examination was taken on the basis of indicators of bioclimatic conditions

prevailing in the same area leading to the method of bioclimatogramme by Uvarov, where the components have average values for temperature and humidity for the studied area. This is of particular importance for assessing the results with respect to embryonation of geohelminths in soil (become infective), under certain conditions like the optimum temperature and humidity.

### 5.3 METHODS OF EXAMINATION OF DOG FECES

The examination is performed using the native preparation method and the flotation method (according to McMaster, Stoll and Richardson-Kendell). Determining the severity of the infection is done by the McMaster method with the application of corrective factors, as well as by the Stoll method with corrective calculation.

### 5.4 SOIL EXAMINATION METHODS

The examination is carried out using the sedimentation and flotation method as well as the modified method according to Pavlovic. The intensity of the infection is determined using the method according to McMaster and Stoll. We presented modified method of testing soil and sand for the presence of parasite eggs according to Pavlovic, protected by the Institute for Intellectual Property of the Republic of Serbia; certificate no. 2770/2017 A-0098/2017 serial no. under which it was entered in the records: 8430.

**Equipment required for work:** 2 laboratory beakers with a volume of 1000 ml, 1 conical sedimentation beaker with a volume of 250 ml, a graduated vessel of 100 ml, a laboratory balance, a physiological solution (protected with 650 g of glucose per liter), a stirring stick, parasitological sieves with an opening size of 250, 150 and 45 micrometers, plastic strainer, pipettes, McMaster counting chamber and microscope with 40x to 100x magnification. **Performance:** - Take 100 g of the sample in a laboratory beaker with a volume of 1000 ml. Pour 500 ml of tap water into and mix the sample and water well - Immediately after mixing, pour the suspension through a parasitological sieve with openings of 250 micrometers into a laboratory beaker with a volume of 1000 ml and leave for 10 minutes to sediment - Drain the liquid and pour 500 ml of tap water on the sediment and mix the sample and water - Immediately after mixing, pour the suspension through a parasitological sieve with openings of 125 micrometers into a laboratory beaker with a volume of 1000 ml and leave for 10 minutes to sediment - Drain the liquid and pour 500 ml of tap water on the sediment and mix the sample and water - Immediately after mixing, pour the suspension through a parasitological sieve with openings of 45 micrometers into a laboratory beaker with a volume of 1000 ml and leave

for 10 minutes to sediment - Measure 4g of sediment with a pipette, transfer it to a conical sedimentation beaker and add 56ml of physiological solution with a graduated vessel - The sample is taken with a pipette immediately after filtering when the suspension is still well mixed. - Pour both sides of the McMaster counting chamber with the suspension, taking care not to create air bubbles, and let it stand for 3-5 minutes before counting. in order for the eggs to settle - In the final stage of McMaster's numerical chamber, it is placed on a microscope slide and examined under a microscope with a magnification of 40-100 x. - When counting the engraved spaces, one should follow the general rule of counting: one should count all the eggs inside the grid plus all the eggs that touch the two sides of the grid (i.e. the upper and left boundary lines), and not take into account all the eggs that touch the other two sides grids (ie lower and right boundary lines). - In McMaster's technique, 15 ml of suspension represents 1 g of sample, and as 0.3 ml is examined, which represents 1/50 g, the total number of eggs in both sides of the chamber should be multiplied by 50.

## 5.5 DETERMINATION OF PARASITES

In both cases, the determination of adults, eggs and oocysts of the parasite is performed by morphometric analysis based on morphological characteristics.

All these years, the researches were organized and carried out in the laboratory for parasitology of Scientific Institute of Veterinary Medicine of Serbia. For easier monitoring of the results, they are shown in average value for five-year periods as we did during the research.

## 6 RESEARCH RESULTS IN THE PERIOD 1993-2023

In order to properly perceive the current situation, some basic facts that preceded it must be known. Until the beginning of the 90s, Belgrade had a negligible population of unowned dogs, and there were legal provisions obliging pet owners to do mandatory deworming, clean public areas after them and responsible ownership. As a result, public areas, parks and green spaces were minimally contaminated with dog feces. At the beginning of the 90s and the breakup of Yugoslavia, there was a state of war in the surrounding area, UN sanctions on the FRY, inflation, etc. These negative events lead to an economic crisis and the consequent abandonment of pets by old owners who could not financially support them. These animals bred freely, and the population of stray dogs grew significantly and became a city problem, as did the contamination of public areas with their feces.

## 6.1 EXAMINATION IN PERIOD 1993-1996

For these reasons, in 1993, the first investigations into the contamination of parks and green areas in Belgrade began. During the first years of research (1993-1996) it was established that 65,90 % of soil samples, 12.5% of sand samples from children's playgrounds and 59.23% of dog feces samples contained parasite eggs. At examined locations eggs of *Toxocara canis* were found in 48,76% soil samples, *Ancylostomidae* sp. in 39,23%, *Dipylidium caninum* in 33.96%, *Trichuris vulpis* in 14.29% and *Taenia* sp. in 7.19%.

At same time in dog faeces eggs of *Toxocara canis* were found in 69.90%, *Ancylostomidae* sp. in 49.96%, *Dipylidium caninum* in 49.62%, *Trichuris vulpis* in 14.99% and *Taenia* sp. in 10.71%. *Isospora* sp. was found in 1,21%, *Giardia intestinalis* in 0.11%, *Amoeba* sp. in 0.02% and *Cryptosporidium* sp. in 0.01%.

The Secretariat for Environmental Protection of Belgrade was informed of the results of the research and measures were taken to control stray dogs and clean children's playgrounds.

## 6.2 EXAMINATION IN PERIOD 1997-2002

In the period 1997-2002, began the arrangement of the city's central park areas, which was reflected in the general reduction of the pollution of sand for children's play to only 0.2% and green areas to 50.22 %. During examination period eggs of *Toxocara canis* were found in 46.56%, *Ancylostomidae* sp. in 37.31%, *Dipylidium caninum* in 33.43%, *Trichuris vulpis* in 10.93%. *Toxascaris leonina* in 7.81% and *Taenia* sp in 6.12%

In dog faeces eggs of *Toxocara canis* were found in 65.90%, *Ancylostomidae* sp. in 41.96%, *Dipylidium caninum* in 44.62%, *Trichuris vulpis* in 11.99% and *Taenia* sp. in 7.71%. *Isospora* sp. was found in 1,11%, *Giardia intestinalis* in 2.32%, *Amoeba* sp. in 1.02% and *Cryptosporidium* sp. in 0.12%.

## 6.3 EXAMINATION IN PERIOD 2003-2007

During the next five years (2003-2007), extensive rehabilitation of park areas and educational activity through the media about responsible ownership and the importance of dog parasites for human health continued. This was done through TV and radio broadcasts, education of children in kindergartens and through leaflets.

The results were already seen during subsequent controls, when contamination was found on 45.90% of the examined surfaces. *Toxocara canis* was found in 42.82% of samples, *Dipylidium caninum* in 37.19%, *Ancylostomidae* sp. in 31.70%, *Taenia* sp. in 4.31%, *Toxascaris leonina* in 3.65% and *Trichuris vulpis* in 2.43%. At same time in dog faeces



eggs of *Toxocara canis* were found in 42.80%, *Ancylostomidae* sp. in 37.19%, *Dipylidium caninum* in 31.62%, *Trichuris vulpis* in 7.92% and *Taenia* sp. in 5.71%. *Isospora* sp. was found in 3.22%, *Giardia intestinalis* in 5.01% and *Amoeba* sp. 3.31% and *Cryptosporidium* sp. in 0.11%.

#### 6.4 EXAMINATION IN PERIOD 2008-2011

In the period 2008 - 2011, visual instructions, notices and markings were made for areas designated for off-leash dogs as well as where dogs are not allowed. In the same period, a system of baskets with PVC bags for the disposal of dog feces (doggy-pot system) was created, and since 2011, eco zones or dog parks have been formed in some parks.

Based on the performed parasitological control of soil contamination from parks, the presence of parasite eggs was found in 39.06%. Eggs of *Toxocara canis* were found in 36.56%, *Ancylostomidae* sp. in 27.31%, *Dipylidium caninum* in 23.43%, *Trichuris vulpis* in 10.93%, *Strongyloides stercoralis* and *Toxascaris leonina* in 7.81% and *Taenia* sp in 6.12%.

In dog faeces eggs of *Toxocara canis* were found in 31.36%, *Ancylostomidae* sp. in 32.50%, *Dipylidium caninum* in 35.62%, *Trichuris vulpis* in 14.99% *Toxascaris leonina* 5.11%, *Strongyloides stercoralis* 4.00% and *Taenia* sp. in 4.19%. *Isospora* sp. was found in 2.32%, *Giardia intestinalis* in 19.37 %, *Amoeba* sp. in 10.11% and *Cryptosporidium* sp. in 5.01%.

The biggest step forward in solving this problem was made in the period 2010-2011. A special segment in Belgrade has been the adoption of problem-solving strategies in the city of Belgrade for stray dogs and cats, which was adopted at the Belgrade City Assembly held 2011. developing this Strategy for the City of Belgrade and its implementation was guided by the principle of humanity. It combines the method of no kill strategy and CNR method “catch - treat - let “ (CNR - catch - neuter -release), with special emphasis on the protection of human and animal health and applying education, control and sanctions against irresponsible owners whose negligence and disregard of legal rules and moral principles directly cause an increase in the number of abandoned dogs. The city of Belgrade has become one of the few cities that has a strategy to solve the problem of stray dogs and defines the principles, objectives and measures to solve the problem of stray dogs by administration of the local government.

#### 6.5 EXAMINATION IN PERIOD 2012-2016

In the period 2012-2016, the trend of building dog parks within city parks continued, the education program on responsible ownership and landscaping of public

areas continued. Control of green areas and parks showed that the percentage of contamination decreased to 30.45%. In this period the average percentage of *Toxocara canis* eggs found was 29.68%, *Ancylostomidae* sp. 21.18%, *Dipylidium caninum* 26.56%, *Strongyloides stercoralis* 6.81%, *Toxascaris leonina* 5.97%, and *Trichuris vulpis* 2.68% and *Taenia* sp. 2.25%.

In dog faeces eggs of *Ancylostomidae* sp. were found in 31.96%, *Toxocara canis* in 29.90%, *Dipylidium caninum* in 25.62%, *Strongyloides stercoralis* in 5.78%, *Toxascaris leonina* 4.91%, *Trichuris vulpis* in 1.39% and *Taenia* sp. in 2.71%. *Isospora* sp. was found in 3.32%, *Giardia intestinalis* in 16.37 %, *Amoeba* sp. in 9.51% and *Cryptosporidium* sp. in 2.01%.

## 6.6 EXAMINATION IN PERIOD 2017-2023

The results indicate that the implementation of this strategy stabilized the population of stray animals and implementation of mass sterilization resulted in a 50% reduced increase of abandoned dogs in full cooperation with all the other organizations with animal welfare goals. The positive effect of these measures was observed during regular parasitological controls of parks and green areas. During 2023, the presence of parasite eggs in public places was >65% less than in the period before the introduction of all these preventive measures and presented it 27.37%.<sup>56</sup>

In dog faeces eggs of *Ancylostomidae* sp. were found in 29.16%, *Toxocara canis* in 28.37%, *Dipylidium caninum* in 27.42%, *Strongyloides stercoralis* in 4.78%, *Toxascaris leonina* in 4.39%, *Trichuris vulpis* in 1.09% and *Taenia* sp. in 1.71%. *Isospora* sp. was found in 1.12%, *Giardia intestinalis* in 22.34 %, *Amoeba* sp. in 9.92% and *Cryptosporidium* sp. in 1.17%.

## 7 METHODS OF REMEDY OF CONTAMINATION

In addition to reducing the number of animals from the streets, it is necessary to undertake a series of measures that are necessary for an adequate solution to this problem on a long-term level. This problem should therefore be approached from several aspects and with the involvement of all structures of the city. This could best be illustrated through the example of the periodic resolution of this problem in Belgrade.

Although the lack of continuity of certain segments has been particularly present in the last few years, past experiences have given us the right that the remediation of this problem is best done when:

1. Regular parasitological control of parks, green areas and children's sand playgrounds and remediation of the established condition
2. By including the media in the actions undertaken
3. By educating the population
4. Legislation
5. Adopting the Strategy for Solving the Problem of unwanted Dogs

### **1. Regular parasitological control of parks, green areas and children's sand playgrounds and remediation of the established condition**

This control is performed periodically and appropriate hygiene measures are taken based on the results. Based on the findings, the sand should be changed by replacing the surface layer to a depth of 6 cm, and in all places in parks and other green areas, the constant cleaning of dog feces should be organized and, if necessary, the contaminated area should be disinfected. In order to monitor the parasitic contamination of parks and other green areas of Belgrade, since 1993, continuous monitoring of their pollution has been carried out. Inspections are carried out in four central city municipalities where there are the most parks in the city.

They resulted in continuous changes in the way of thinking and contributed to the implementation of numerous changes within the city's communal hygiene, which led to a significant improvement in the quality of these areas and the constant improvement of its protection and thus the health of the people who live here.

### **2. Media in the actions undertaken**

During the actions, the media should be engaged in order to support and acquaint the population with the importance of the implemented actions. The media should influence the propagation of these actions and influence the inclusion of as wide a population as possible in it. Media actions must be conducted in such a way that they do not cause negative reactions from animal protection societies and the like.

Organizations that know how to permanently hinder progress in this industry. In this way, the activists of those groups should be drawn into the normal framework of action in order to cancel their unprofessional actions. In this way, they will focus on a much more cooperative approach to solving the problem of non-owner dogs and allow their care in private shelters to be under the control of veterinarians, as well as the care of animals that are on the street. Eg. activities in this direction, which were carried out in the period 2011-2012, gave a lot of positive progress and in many ways helped to implement the adopted strategy of the city for taking care of stray dogs.

### **3. Education the population**

Perhaps one of the key segments is the education that should include dog owners, considering the large amount of ignorance of the possible role that their pets play in the spread of certain diseases transmitted by dogs, which in case of infection of pets, they are primarily at risk. In this way, their irresponsible behavior and letting dogs onto green areas and children's playgrounds will be avoided or at least reduced. They should also be reminded of the importance of periodic parasitological examinations and deworming of pets. The owner's opinion that taking dogs into the vicinity of the house is not dangerous is completely wrong, because the eggs of these parasites are brought into the house through the shoes and dirty hands of the owners who have been in contact with other dogs.

The education of children in pre-school and school institutions is also of particular importance, considering that a large number of them have a dog as their pet or come into contact with stray animals. Children should be introduced to the behavior of dogs, emphasis should be placed on washing hands after playing with dogs and in the park or on the sand, etc. but also with the potential dangers that threaten them from dogs (injuries, infections, etc.). This should be presented in an accessible and easy way for children, and positive examples were teaching children in kindergartens and taking children to the first open asylum for dogs where they had contact with animals with explanations adapted to their age.

The education of parents of children is even more important, and to this end lectures by experts should be held simultaneously for children and parents, especially in preschool institutions.

Education of health workers (pediatricians), considering that the problem of larva migrans and other parasitoses is not yet given enough attention, should be educated through seminars, which would involve them equally in the action of educating patients.

The education of veterinarians, primarily those engaged in small practices, should also be carried out through seminars. Their work should also be focused on the education of owners in controlling helminths and the need for regular coprological control of pet dogs.

To that end, this problem should be presented in several ways. First, it is necessary to start issuing posters, leaflets and brochures that will deal with this problem in an accessible way (in pictures and words). Care should be taken to issue publications in which this problem is brought closer to the population in a clear and concise manner with an appropriate textual and graphic solution.

Education should also be carried out through the media - radio, television and print. Depending on the level of the magazine, the texts should be adapted so as to point out all the actuality, importance and measures that should be taken for the purpose of preventing infections (hygiene of keeping dogs, deworming, obligations of the owner in relation to walking dogs, the possibility and way of infection of children and the need for personal hygiene etc.). The same concept should be followed when presenting on this topic in radio and TV shows, with the fact that veterinarians and doctors should also be included in them.

#### **4. Legislation**

Legislation related to animal welfare, keeping and walking dogs and other regulations related to communal hygiene should be rigorously implemented with all punitive measures against the owners. For that, there is now a municipal police force that should work in accordance with its powers.

#### **5. Adopting the strategy for solving the problem of unwanted dogs**

The city of Belgrade is one of the few cities that has a strategy for solving the problem of unowned dogs, and this is a document that defines the principles, goals and measures that will be implemented by the local self-government in 2011 in terms of solving the problem of unowned dogs. The strategy for solving the problem of stray dogs and cats in the territory of the city of Belgrade is a document that defines the principles, goals and measures that will be implemented by the City of Belgrade in terms of solving the problem of stray dogs. The strategy is monitored and supervised by the Secretariat for Communal Housing Affairs, while the direct executor is VUVB. Operational measures in this area are adopted by the Commission for the implementation of the strategy, which was formed by the conclusion of the mayor. The aforementioned strategy was adopted at the Assembly of the City of Belgrade held on September 21, 2011, based on Article 54, Paragraph 2 of the Law on Animal Welfare, Article 20 of the Law on Local Self-Government, and Articles 25 and 31 of the Statute of the City of Belgrade.

The development of this strategy for the City of Belgrade and its implementation was guided by the principle of humanity, combining the no kill strategy and the CNR method “catch-neuter-release”, with a special focus on health protection people and animals, and applying measures of education, control and sanctions against irresponsible owners whose carelessness and disregard for positive legal regulations and moral principles directly cause an increase in the number of abandoned dogs.

Taking into account the gravity of the problem as well as the fact that solving it directly manipulates animals that are able to feel suffering, fear, stress and pain, it was necessary to objectively look at all the relevant causes of the problem, but also to define measures and methods that will be the most adequate, sustainable and humane way in a time period of 5 to 10 years, the intensive implementation of the Strategy will give results that will:

1. Reduce the population of abandoned animals to an acceptable level, without disturbing the biological balance, the violation of which can cause the growth of the population of other animal species (cats, harmful rodents, etc.);
2. Maintaining the population within the number that ensures biological balance;
3. Providing captured abandoned animals with adequate veterinary assistance, including depriving them of their lives in a humane way, when necessary.

## 7.1 GENERAL GOALS OF THE STRATEGY

During the development of the strategy, the objectives were first determined, which were the starting point for its development. At the sessions that followed, the general goals of the “Strategy for dealing with stray dogs in the territory of the City of Belgrade” were adopted and they are:

1. Reduction of the population of stray dogs and cats;
2. Control of the existing population of dogs and cats;
3. Protection of human and animal health
4. Promotion of responsible animal ownership and welfare
5. Compliance with legal and by-laws
6. Prescribing measures to stimulate sterilization and marking of owner’s animals for population control
7. Construction of new capacities for housing abandoned animals In order to achieve better operability; specific objectives of the strategy were defined along with them.

## 7.2 SPECIFIC OBJECTIVES OF THE STRATEGY

The specific objectives of the strategy were as follows:

1. Registration of all owned dogs and cats in the territory of the city of Belgrade, registration of all abandoned dogs and cats, as well as the formation of a unique database within the database of the Veterinary Administration (vet up).
2. Introduction to the legal status of guardians of street animals

3. Introduction and collection of local communal tax for keeping pets
4. Construction of ecological zones or parks for dogs
5. Development of an action plan, i.e. a control program to reduce the population of stray dogs and cats.

### 7.3 PROBLEMS IN IMPLEMENTING THE STRATEGY

The problems encountered in the implementation of the Strategy, in addition to the harsh economic conditions, are: non-compliance with applicable laws, lack of conditions for the application of existing legal solutions, but also the fact that certain legal solutions are not good enough and need to be corrected. This applies in particular to:

1. Inconsistency of legal regulations, namely the Law on Veterinary Medicine and the Law on Animal Welfare
2. Centralization of control of both above-mentioned laws in the veterinary inspection
3. The absence of an adequate regulation on the marking of dogs and cats, as well as the lack of adequate control and sanctions against irresponsible owners
4. Inadequately prescribed measures, rights and powers of the control agents (veterinary inspection, communal inspection, communal police)
5. Non-recognition through acts of the existence of a street population of abandoned animals and therefore the inability to adequately record, monitor the epizootic situation as well as adoption.

This also prevented the connection of stray dogs and cats to the single database of the Veterinary Administration.

6. Non-recognition of the existence of individuals who want to take care of abandoned animals (guardians), which makes it impossible to keep records of those persons and the number and structure of the animals they take care of.
7. Non-hyphenation of the problem and the term “hoarding”, which represents an almost unsolvable problem, especially in urban areas.
8. The general disinterest of pet owners both in terms of the health care of their own pets (vaccination, chipping, deworming, prevention of unwanted mating), and in terms of environmental protection. The explanation of this phenomenon lies primarily in the ineffective control of the competent authorities, in the lack of authority and in the excessive procedure that is required to adequately punish irresponsible owners.

## 7.4 MONITORING THE EFFECTS OF IMPLEMENTING THE STRATEGY

In order to assess the exact state of affairs and prepare this Strategy, the Project “Determining the population size and category of stray dogs in the territory of the city of Belgrade in urban and suburban areas” was carried out. Based on data from the Project from 2006, as well as data from the Veterinary Institution “Veterina Beograd”, and before the establishment of the “Strategy for solving the problem of stray dogs in the territory of Belgrade” in the 10 central city municipalities, an estimated number of 4,400 to 4,600 dogs with predicted degree of statistical error from 5% to 10%.

By applying the logical matrix of the actual number of street dogs in 2010, it was determined that their number was 8,500 individuals in 10 Central City Municipalities. It could also be concluded that the estimated raw number of 2954 dogs according to the logical matrix, on an annual basis, could produce more than 2250 offspring in the F1 generation alone. With the application and implementation of the “Strategy for Solving the Problem of Stray Dogs in the Territory of Belgrade” in the period of 2009 and 2010, followed by a controlled, precise counting of dogs, using the field method, in the territory of the 10 central city municipalities, the number of 8,500 dogs was determined, with the application euthanasia only and exclusively in medically indicated cases.

## 7.5 RESULTS OF STRATEGY APPLICATION

Analyzing the obtained data, it is unambiguously concluded that:

1. With the implementation of the Strategy for solving the problem of stray dogs in the territory of Belgrade, the population of abandoned animals on the streets was stabilized with a tendency to decrease with the application of the CNR system;
2. By applying the NO KILL (no euthanasia) component of the CNR system, the effect of the increase in the dog population as part of the active implementation of mass sterilization in 2010 resulted in a 50% smaller increase in abandoned dogs;
3. The influx of dogs by irresponsible owners, based on the assessment, constitutes 2/3 of the total number of increments from point 2;
4. In addition to the measures implemented by the Secretariat for Communal and Housing Affairs and the “Veterina Beograd” Veterinary Institution, it is necessary to implement a clear penal policy towards irresponsible owners as well as other measures defined by the Strategy for solving the problem of stray dogs in the territory of Belgrade;



5. It is necessary to increase capacities with the aim of more active performance of animal hygiene activities in the area of other city municipalities;

In addition, based on the results obtained so far, it can be unambiguously concluded that:

1. With the implementation of the Strategy for solving the problem of stray dogs in the territory of Belgrade, the population of abandoned animals on the streets was stabilized with a tendency to decrease with the application of the CNR system;
2. By applying the NO KILL (no euthanasia) component of the CNR system, the effect of the increase in the dog population as part of the active implementation of mass sterilization in 2010 resulted in a 50% smaller increase in abandoned dogs;
3. The influx of dogs by irresponsible owners, based on an estimate, accounts for 2/3 of the total number of abandoned dogs;
4. In addition to the measures implemented by the Secretariat for Communal and Housing Affairs and the “Veterina Beograd” Veterinary Institution, it is necessary to implement a clear penal policy towards irresponsible owners as well as other measures defined by the Strategy for solving the problem of stray dogs in the territory of Belgrade;
5. It is necessary to increase capacities with the aim of more active performance of animal hygiene activities in the area of other city municipalities;

## 7.6 IMPACTS OF THE STRATEGY ON THE CONTAMINATION OF PUBLIC PLACES

At the same time as the adoption of the Strategy, regular parasitological monitoring of public places and parks in Belgrade continued. By the way, he has been working since 1993 [3,5,6,7]. The average contamination of the parks in 2010 was 39.06%, and in 2020 it will drop to 26.68%. During these ten years, the average percentage of established eggs of *Toxocara canis* was 29.68%, *Ancylostomidae* sp. 27.18%, *Dipylidium caninum* 26.56%, *Strongyloides stercoralis* 7.81%, *Toxascaris leonina* 6.97%, *Taenia* sp. 6.25% and *Trichuris vulpis* 4.68%. *Giardia duodenalis* was found in 15.62%, *Amoeba* sp. in 11.06%, *Isospora* spp. in 9.36% and *Cryptosporidium* sp. in 3.37%. A visible reduction in the degree of contamination of public places was observed in the mentioned period. Despite the visible improvement, from an epidemiological point of view, the research results indicate a real possibility of human infections in the areas of most city parks.

## 8 CONCLUSIONS

Public interest in the welfare of animals is growing and is increasingly present in the world, as well as in our society. This trend inspires numerous debates and discussions about animal welfare as well as ways and problems that arise when solving the problem of abandoned animals. Factual and scientific knowledge in this area must support and inform ethical decisions, and especially the need to understand the affective states of animals (the problem of interaction with people) and their needs in relation to the environment in which they move and find themselves. Animal welfare is a course that should guide us in applying methods to solve the problem of abandoned animals. The positions that need to be defended, which concern the welfare of animals, require scientific knowledge about the biology of animals, in order to enable the closest determination of their physiological, health and psychological needs, as well as to better solve the problems and issues of their impact on the environment and people's health. The bearers of solutions in this area should first of all be veterinarians, who will bring programs and give expert opinions, and then all state and public officials, who can contribute to a successful solution to this problem through their work and advocacy.

With this approach, our society ranks among developed countries, which approach the solution to the problem of abandoned animals on a scientific basis, and the quality of the Strategy itself is best expressed by the fact that similar guidelines are given in the European Parliament Written Declaration pursuant to Rule 123 of the Rules of Procedure on dog population management in the European Union No 0026/2011 of 06.10.2011 (6) (adopted almost a month after the adoption of our Strategy). This Strategy has also shown positive results in reducing the pollution of public places by dog parasites because it contributes to the preservation of human health.

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# CAPÍTULO 15

## DISEÑO CONCEPTUAL DE UN SISTEMA DE PRODUCCIÓN DE ALIMENTOS BALANCEADOS PARA GANADO PORCINO<sup>1</sup>

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**RESUMEN:** La investigación se centra en el diseño de una máquina trituradora y peletizadora destinada a la producción de alimentos para ganado porcino, abordando un problema crítico que enfrentan los poricultores rurales. Estos productores han visto un aumento del 150% en los precios de los productos balanceados, lo que les impide adquirirlos debido a sus limitados recursos económicos. La solución propuesta consiste en permitirles fabricar sus propios alimentos utilizando insumos como maíz y otros cereales. Para el desarrollo del diseño conceptual, se aplica la metodología de Pahl y Beitz, junto con diversas herramientas de diseño. A través del modelo KANO, se identifican atributos clave como eficiencia, estética, multifuncionalidad, fácil operación y economía, que deben ser considerados en el diseño. Estos atributos se convierten en características específicas que guían la creación de tres bocetos mediante la matriz morfológica de Zwicky. La selección del diseño final se realiza utilizando el método de evaluación de objetivos ponderados,

<sup>1</sup> Se agradece a los estudiantes de la ingeniería en Metalmecánica del grupo A de la generación 2020-2023, por la colaboración activa en la recolección de datos a través de las encuestas aplicadas.

resultando en la elección del boceto B, que obtuvo una puntuación de 3.5, destacándose por su facilidad de operación y multifuncionalidad. La conclusión del estudio es la propuesta de una máquina que incorpora un dosificador mediante un mecanismo sinfín. Para futuros desarrollos, se sugiere implementar un sistema deshumidificador para el alimento procesado y realizar pruebas con carga, ya que hasta ahora solo se han llevado a cabo pruebas sin carga con resultados satisfactorios.

**PALABRAS CLAVES:** Atributos. Diseño. Triturado. Peletizado. Porcino.

## CONCEPTUAL DESIGN OF A BALANCED FOOD PRODUCTION SYSTEM FOR SWINE

**ABSTRACT:** The research focuses on the design of a grinder and pelletizer machine for the production of swine feed, addressing a critical problem faced by rural pig farmers. These producers have seen a 150% increase in the prices of balanced products, which prevents them from acquiring them due to their limited economic resources. The proposed solution is to enable them to manufacture their own feed using inputs such as corn and other cereals. For the development of the conceptual design, the Pahl and Beitz methodology is applied, together with various design tools. Through the KANO model, key attributes such as efficiency, aesthetics, multifunctionality, ease of operation and economy are identified, which must be considered in the design. These attributes are converted into specific characteristics that guide the creation of three sketches using Zwicky's morphological matrix. The selection of the final design is made using the weighted objective evaluation method, resulting in the choice of sketch B, which obtained a score of 3.5, standing out for its ease of operation and multifunctionality. The conclusion of the study is the proposal of a machine that incorporates a dispenser by means of an auger mechanism. For future developments, it is suggested to implement a dehumidifying system for the processed feed and to carry out tests with load, since so far only tests without load have been carried out with satisfactory results.

**KEYWORDS:** Attributes. Design. Grinding. Pelleting. Swine.

### 1 INTRODUCCIÓN

Actualmente, México ocupa el lugar 13 como productor de carne porcino en el mundo, cada año se comercializan más de 250 mil toneladas de productos de cerdo en países como Japón, Estados Unidos, Singapur, Canadá, Corea del Sur Vietnam, Hong Kong, Chile y China. (SADER, 2022).

Según las expectativas del Grupo Consultor de Mercados Agrícolas (GCMA, 2022) estima que el consumo del sector pecuario alcanzará los 30 millones de toneladas 3.3% más que el año anterior. El crecimiento se atribuye a un mayor consumo de carne de puerco que pasará de 2.75 MT en 2021 a 2.83 MT en 2022 (incremento del 3%).

De acuerdo a SENASICA (2022), establece que en el sector agroalimentario a pesar de las pandemias; incremento de costos de los insumos básicos; bienes públicos

insuficientes, la administración de riesgos, se sigue enviando alimentos sanos e inocuos a la mesa de millones de familias en México y el mundo.

Sin embargo, dentro de esta actividad los pequeños productores enfrentan problemas en el proceso de la alimentación de porcinos y se ha convertido en una actividad alternativa válida para los pequeños productores de cerdos y que influye directamente en los gastos de operación en términos económicos representando el 69.4 % (SENASICA, 2021. pp. 8). Sin embargo, la problemática productiva, influye directamente en los bajos rendimientos, por lo que es el resultado de una serie de factores que influyen negativamente en el proceso productivo y entre los cuales los más evidentes son: la inadecuada alimentación de los nutrientes y al deficiente control de costos de la actividad (CISP, 2009 como citado en Calderón, 2012).

Ante el constante aumento de los precios de alimentos balanceados para cerdos, cada vez la rentabilidad de la actividad se ha ido disminuyendo para los pequeños productores, además de que no cuentan con suficiente recurso económico debido a sus ingresos bajos percibidos (Rivas, 2021).

Con el incremento de la demanda local de alimento, según Morales (2010), el cerdo sigue siendo una alternativa clave dentro de la problemática de cualquier sistema de producción integrado, por ser un animal que presenta una serie de ventajas derivadas de su capacidad de adaptarse fácilmente a diferentes esquemas de manejo y alimentación, con la característica de ser en ciertos casos el perfecto reciclador dentro de un sistema pecuario; por lo que pueden generarse nuevas formulaciones de alimentos a través de la disponibilidad de otras materias primas en cada región donde se requiera.

Por lo tanto, se plantea la realización del diseño conceptual de una máquina para un sistema de producción de alimentos balanceados, a mediante técnicas de diseño y desarrollo de nuevos productos.

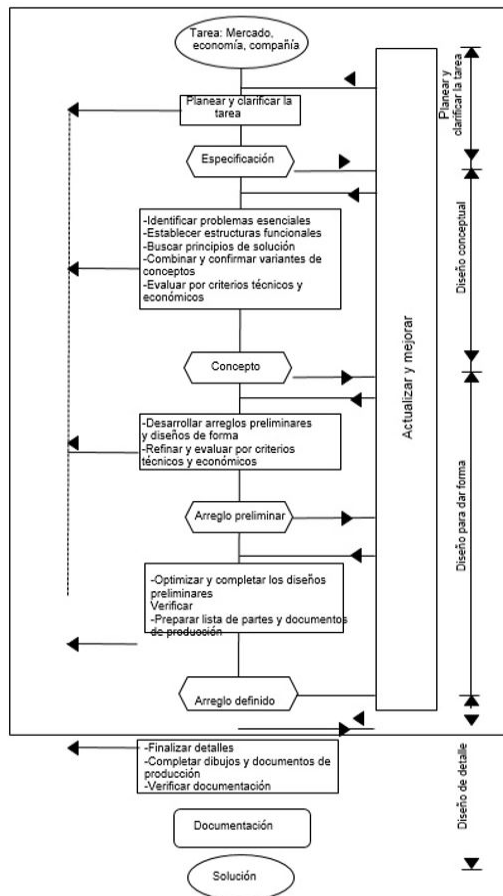
## 2 METODOLOGÍA

El desarrollo de este artículo se realizó mediante la metodología de diseño de Pahl y Beitz, en este modelo el proceso de diseño es compuesto por cuatro fases principales como son: planear y calificar, diseño conceptual, diseño para dar forma y diseño de detalle, que se describe a detalle en la figura 1 (Pahl y Beitz, 1996), y que se describe el proceso en este apartado del artículo.

## 2.1 CARACTERIZACIÓN DEL PROCESO EN EL SISTEMA DE PRODUCCIÓN DE ALIMENTOS

El pelletizado del alimento es un proceso que permite que pequeñas partículas vegetales, principalmente, se aglomeren en gránulos compactos a través de la acción de los rodillos y dados para su conformación. Esto es un proceso con elevados costos por consumo de energía, pero que se justifica con el rendimiento que imprime al sector pecuario (Meinerz, 2001, citado en Salcido 2017, pág. 9).

Figura 1. Pasos en el proceso de planificación y diseño a través del Modelo prescriptivo del diseño de Pahl y Beitz.



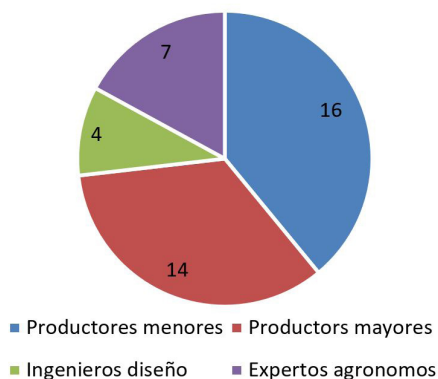
Fuente: Diagrama tomado del libro: Pahl Beitz Engineering Design A Systematic Approach pág. 130.

## 2.2 APLICACIÓN DEL MODELO KANO

El proceso del modelo kano, se procede a la identificación de las características iniciales propuestas del sistema de producción de alimentos balanceados para ganado porcino.

Se aplicaron un total de 41 encuestas, de las cuales en la siguiente gráfica se indica la distribución de los encuestados.

Figura 2. Distribución de las encuestas aplicadas.



Fuente: Elaboración propia.

De acuerdo a la encuesta aplicada, y una vez recolectados y cuantificados las respuestas se muestra las características clasificadas por atributos definidos para el modelo kano.

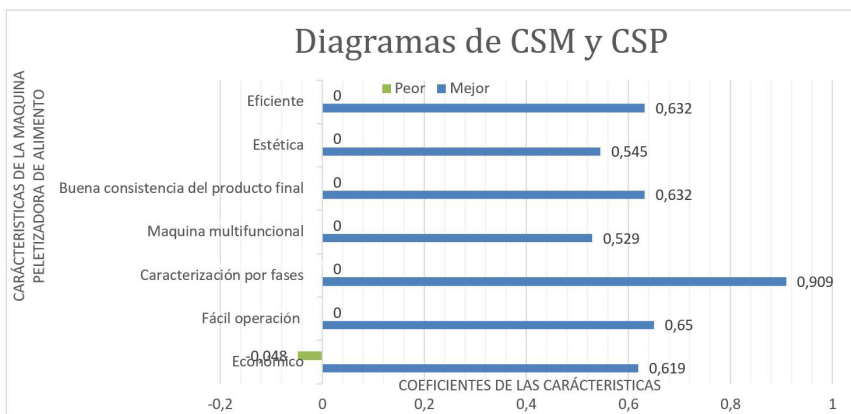
Tabla 1. Resultado de los encuestados.

Núm.	Atributos definidos para la maquina pelletizadora de alimento	Atributo Atractivo	Atributo Obligatorio	Atributo Opuesto	Atributo Unidimensional	Atributo Dudoso	Atributo Indiferente	Total	Calificación	Nivel de importancia	Mejor	Peor	C1	C2	Q	abs(a-b)
		A	O	R	U	D	I	Total								
1	Económico	12	0	0	1	2	8	23	I	0.891	0.619	-0.05	0.551	0.043	3.273	7
2	Fácil operación	13	0	1	0	3	7	24	U	0.875	0.65	0	0.569	0	3.12	-13
3	Caracterización por fases	20	0	0	0	2	2	24	D	0.974	0.909	0	0.885	0	3.053	2
4	Maquina multifuncional	9	0	0	0	7	8	24	A	0.859	0.529	0	0.455	0	3.23	9
5	Buena consistencia del producto final	12	0	2	0	3	7	24	U	0.891	0.632	0	0.563	0	3.273	-7
6	Estética	12	0	0	0	2	10	24	U	0.87	0.545	0	0.474	0	3.179	-10
7	Eficiente	12	0	0	0	1	7	20	U	0.88	0.632	0	0.556	0	3.23	-7

Fuente: Elaboración propia.

De acuerdo a la tabla anterior se nota que existen cuatro características como atributos unidimensionales, una característica como atributo atractivo, una característica como atributos indiferentes y una característica como atributo dudoso. Ninguna característica se clasifico como obligatorio. Para el diseño conceptual se utilizará como características para el diseño del sistema productor de alimento balanceado para ganado porcino los atributos atractivos, unidimensionales e indiferentes.

Figura 4. Grafica de mejor y peor atributo del modelo KANO.































Fuente: Elaboración propia.

En esta imagen se muestra los atributos mejores y peores evaluados de acuerdo al modelo kano, por lo que se logra apreciar que el atributo menos esencial es el económico ya que es el peor evaluado.

### 2.3 APLICACIÓN DE LA MATRIZ MORFOLÓGICA

El objetivo del método es generar un conjunto de soluciones alternativas de diseño conceptual de la máquina pelletizadora de alimento para ganado porcino, y por lo tanto ampliar la investigación para nuevas soluciones potenciales.

Figura 5. Propuestas de solución.

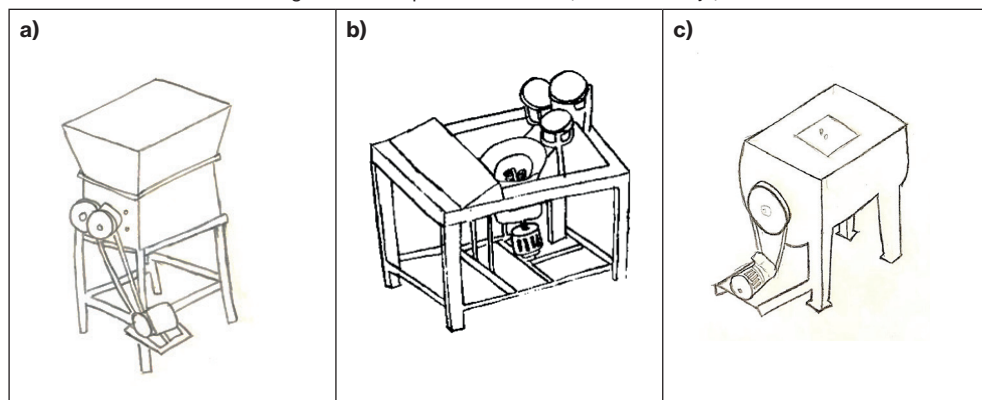
Funciones	Propuestas de solución				
	Alternativa 1	Alternativa 2	Alternativa 3	Alternativa 4	Alternativa 5
Fuente de alimentación	 Motor monofásico	 Motorreductor	 Motor Trifásico		
Controlador de suministro de energía	 Interrupción momentánea rojo y verde	 Interrupción termomagnética			
Transmisión de potencia	 Sprocket con cadena	 Polea y Banda	 Sistema de engranes	 Sistema de poleas	
Traslado de la máquina	 Deslizable	 Elementos desmontables	 Remolcada por un tirón	 Ruedas	
Operación de triturado	 Molino de martillos	 Molino de cuchillas	 Pellets	 Molino de corte	 Molinos de disco
Unión y conexiones	 Tornillos y tuercas	 Soldadura	 Remaches		
Introducción del alimento	 Tolva	 Manual			
Descarga de alimento	 Banda transportadora	 Por ducto	 Manual		
Sistema de control	 Operador humano	 Inteligencia artificial			

## 2.4 ANÁLISIS DE LOS CONCEPTOS

A continuación, se presenta la comparación de las distintas propuestas de conceptos generados y así con la finalidad de lograr obtener aquel que mejor satisfaga los requerimientos del cliente.



Figura 6. Concepto de solución A (Línea color Rojo).



Fuente: Elaboración propia.

El Concepto de solución A (Línea color Azul) de la figura 6b, se emplea un motor monofásico como fuente de alimentación de potencia. La materia prima será suministrada a través de una tolva, después caerá a la operación de triturado que estará encargado por un molino de cuchillas, posteriormente el alimento pasará en un ducto de salida y caerá en un recipiente. Además, el controlador de suministro de energía será encargada por un interruptor termomagnético, la transmisión de potencia se llevará a cabo por un sistema de engranes y contará con unas ruedas para su fácil transportación.

El Concepto de solución C (Línea color Negro) de la figura 6c, será accionada por un motorreductor que transmitirá potencia por medio de poleas y banda, la materia prima será colocada de forma manual dentro de un contenedor y posteriormente caerá por gravedad dentro de la tolva que estará conectada directamente hacia la cámara de aglutinado, en donde se comprimirá el alimento entre una matriz plana y un par de rodillos de compresión, los cuales compactaran las partículas de harina hasta formar los pellets, el cual ya formado caerá por un ducto de salida y se dirigirá dentro de un contenedor. Además, se utilizaras interruptores momentáneos rojos y verdes quienes serán encargados de controlar el suministro de energía de la máquina.

El diseño conceptual C, se emplea un motor trifásico como fuente de alimentación de potencia. La materia prima será suministrada de manera manual y caerá directamente a la operación de triturado, en donde con una lluvia de golpes de martillo la materia prima será golpeado por varios martillos que estarán unidos a un eje que girara a altas velocidad, dentro de una cámara de aglutinado. El alimento se tritura por impacto de los martillos repetidas veces hasta conseguir la consistencia deseada y posteriormente pasará por una matriz y el alimento será extraído por una banda trasportadora y será llevado hacia un contenedor.

Además, se utilizará interruptores termomagnéticos para el control del suministro de energía, la transmisión de potencia estará basada en el sistema de engranes que ayudará a una mejor.

## 2.5 MÉTODO DE OBJETIVO PONDERADOS. EVALUACIÓN DE CONCEPTOS

En esta parte se evalúan las propuestas de diseño de los conceptos generados, con la finalidad de lograr obtener aquel que mejor satisfaga los requerimientos del cliente. Para así tener un claro concepto de diseño.

En este método se realizó un análisis cuantitativo en el que se evaluaron entre si los diferentes conceptos de diseño y así obtener una solución óptima de diseño para la fabricación de la peletizadora. Para la selección del concepto se han considerados atributos que se obtuvieron con el modelo Kano y así se le asignó un peso relativo a cada uno y posteriormente a eso se hizo una multiplicación del peso por la calificación de cada uno de los atributos y se dividió entre 100%.

Tabla 2. Evaluación de conceptos de diseño.

N°	Evaluación de conceptos Atributos	Peso	Concepto A		Concepto B		Concepto C	
			C	EP	C	EP	C	EP
1	Económico	5	4	0.2	3	0.15	3	0.15
2	Fácil operación	13	4	0.52	4	0.52	4	0.52
3	Caracterización por fases en la maquina	25	2	0.5	4	1	2	0.5
4	Máquina multifuncional	8	2	0.16	3	0.24	3	0.24
5	Buena consistencia del producto final (tamaños normalizados)	20	3	0.6	3	0.6	3	0.6
6	Estética	12	2	0.24	4	0.48	4	0.48
7	Eficiente	17	4	0.68	3	0.51	3	0.51
	<b>Total</b>	100		2.9		3.5		3

Fuente: Elaboración propia.

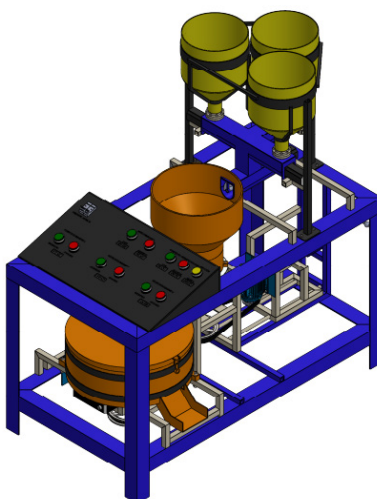
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Los atributos considerados son, económico, fácil operación, caracterización por fases en la máquina, máquina multifuncional, buen producto, estética y eficiente. La

propuesta A obtuvo una ponderación de 2.9 el concepto B, logro una puntuación de 3.5 y finalmente el concepto C obtuvo una ponderación de tres. Después de haber realizado la ponderación se llegó a la conclusión de que la alternativa óptima para el desarrollo de este proyecto es la alternativa B, debido a que es el concepto con la máxima puntuación de las 3 evaluadas, y de cual tiene una fuerte relación con los requerimientos del cliente expresadas en atributos de diseño. Una vez obtenido el concepto se procede a realizar el modelo tridimensional en el software Solid Works.

## 2.6 CONCEPTO DE DISEÑO

Figura 7. Diseño 3D de la maquina peletizadora de alimento para ganado.



Fuente: Elaboración propia.

El modelo de diseño propuesto de la pelletizadora de alimento estará compuesto por dos motores, uno de ellos estará encargado de proporcionar movimiento a eje de las ruedas martillo y el otro al deshumidificador de alimento, además también contará con cuatro servomotores NEMA 17 con capacidad en torque de 3Nm en la parte del dosificador de los tres alimentos.

## 3 RESULTADOS Y DISCUSIÓN

El modelo de diseño de Pahl y Beitz se utilizó como metodología para el desarrollo de este proyecto, a base de eso en la etapa de fase conceptual se aplicó el modelo Kano en la cual se obtienen los siguientes resultados, existen cuatro características como atributos unidimensionales, una característica como atributo

atractivo, una característica como atributos indiferentes y una característica como atributo dudoso. Ninguna característica se clasificó como obligatorio. Para el diseño conceptual se utilizará como características para el diseño del sistema productor de alimento balanceado para ganado porcino los atributos atractivos, unidimensionales e indiferentes. Posteriormente se elaboraron tres conceptos diferentes a través de la matriz morfológica que incluían los atributos obtenidos a través del modelo KANO y se seleccionó uno utilizando el método de objetivos ponderados resultando que el concepto B obtuvo una ponderación de 3.5 contra el 2.9 y 3 los conceptos A y C, respectivamente. La máquina pelletizadora contará con tres contenedores en donde se abastecerá el alimento con una capacidad de en cada uno. Además, tiene unas dimensiones de 1.60m de alto x1.50m de largo x0.8 m de ancho.

De acuerdo al análisis de cargas para las columnas con cargas concéntricas de los portacontenedores, la carga crítica que puede provocar una deflexión lateral, es una carga de 20.194 kN, sin embargo, ninguno de las columnas que soportan los silos contenedores alcanza esta carga ya que la máxima de las tres es de 333 N que corresponde al silo del Núcleo. Para el caso del análisis de las tres columnas con cargas excéntricas, la primera columna es la que soporta al silo del maíz, donde se aplica la carga total es de 219 N, se genera un esfuerzo máximo de 111 MPa, y siendo que el esfuerzo de fluencia del acero ASTM A36 es de 250 MPa por lo que se protege un con factor de seguridad de 2.25. La segunda columna corresponde al silo de la soya, donde se aplica la carga de 227 N, se genera un esfuerzo máximo de 115 MPa, por lo el factor de seguridad es de 2.17 Por último, la tercera columna corresponde al silo del núcleo que soporta una carga de 333 N y genera un esfuerzo máximo en la columna de 169 MPa y de esta manera es seguro con un factor de seguridad de 1.47.

De esta manera se concluye que ninguna de las tres columnas va fallar por fluencia ni por carga crítica, aunque se tiene presente se puede optimizar la sección transversal de las columnas sin arriesgar la integridad de la misma ni aumentado los costos de fabricación ya que se tienen que respetar la voz del cliente obtenido a través del modelo KANO.

## 4 CONCLUSIONES

Este artículo se presentó el diseño conceptual de una máquina pelletizadora de alimento para ganado porcino quien constará con un dosificador de alimento y esto estará en función de gusanos transportadores quienes llevarán el alimento hacia el proceso de pelletizado, también poseerá un sistema deshumificador ya que el alimento

al ser pelletizado saldrá con una constancia húmeda y este sistema ayudara a retirar la humedad de los pellets.

Con los cálculos realizados se llegó a conocer la cantidad de alimentó que podrá almacenar cada contenedor y las dimensiones de la maquina peletizadora de alimentó. Para las dimensiones del pellet dependerá del tamaño de la matriz de este sistema, cabe recalcar que los alimentos en forma de pellets ayudan a evitar la pérdida de los nutrientes del alimento, disminuye el desperdicio del alimentó, destruye organismos patógenos y además mejorara la palatabilidad y digestibilidad del alimento.

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## SOBRE O ORGANIZADOR

**EDUARDO EUGENIO SPERS** realizou pós-doutorado na Wageningen University (WUR), Holanda, e especialização no IGIA, França. Possui doutorado em Administração pela Universidade de São Paulo (USP). Foi Professor do Programa de Mestrado e Doutorado em Administração e do Mestrado Profissional em Comportamento do Consumidor da ESPM. Líder do tema Teoria, Epistemologia e Métodos de Pesquisa em Marketing na Associação Nacional de Pós-Graduação e Pesquisa em Administração (ANPAD). Participou de diversos projetos de consultoria e pesquisa coordenados pelo PENSA e Markestrat. É Professor Titular no Departamento de Economia, Administração e Sociologia, docente do Mestrado em Administração e Coordenador do Grupo de Extensão MarkEsalq no campus da USP/Esalq. Proferiu palestras em diversos eventos acadêmicos e profissionais, com diversos artigos publicados em periódicos nacionais e internacionais, livros e capítulos de livros sobre agronegócios, com foco no marketing e no comportamento do produtor rural e do consumidor de alimentos.

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